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Why do Arctic Grayling (*Thymallus arcticus*) Get Bigger as You Go Upstream?

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Hughes, N.F., and J.B. Reynolds. 1994. Why do Arctic Grayling (*Thymallus arcticus*) get bigger as you go upstream? Can. J. Fish. Aquat. Sci. 51: 2154–2163.

During the summer Arctic grayling in interior Alaskan streams get bigger as you go upstream. We performed a fish removal experiment in a small stream to test two hypotheses about the mechanism that produces this size gradient. The first hypothesis was that all sizes of Arctic grayling prefer positions in the headwaters, but that large fish defend these, forcing smaller ones to occupy positions further downstream (the competition for ranked positions hypothesis). The second hypothesis was that small Arctic grayling actually prefer positions in downstream reaches, and that as they grow their habitat preferences change, and they move to positions further upstream (the size-dependent habitat preference hypothesis). Under this hypothesis, exclusion by other individuals plays no part in producing the whole-stream size gradient. Using the distribution pattern of fish immediately before the removal experiment we developed two sets of rules, one for each hypothesis, to predict how a smaller post removal population, with a different size structure, should be distributed. By comparing these predictions with the actual distribution pattern of the population two years after the removal, we found that the competition for ranked positions hypothesis made much more accurate predictions than the alternative.

Durant l'été, les ombres arctiques trouvés dans les cours d'eau de l'intérieur de l'Alaska sont de plus en plus gros à mesure qu'on remonte en amont. Nous avons procédé à un enlèvement expérimental des poissons dans un petit cours d'eau afin de tester deux hypothèses susceptibles d'expliquer ce gradient de taille. La première, c'est que les ombres arctiques de toute taille préfèrent être situés en amont, mais que les poissons les plus gros occupent ces territoires et les défendent, forçant de la sorte les plus petits à occuper des territoires situés plus en aval (l'hypothèse d'une compétition pour occuper une position relative). La seconde hypothèse, c'était que les ombres plus petits préfèrent être situés dans les tronçons situés en aval et qu'à mesure qu'ils grossissent, ils se mettent à préférer les habitats situés plus en amont et s'y dirigent progressivement (l'hypothèse d'une préférence d'habitat selon la taille), selon cette hypothèse, il n'y a aucun effet sur le gradient qui soit exercé par exclusion de sujets par de plus gros. En s'appuyant sur la distribution des poissons immédiatement avant l'expérience, nous avons créé deux ensembles de règles, l'un pour chaque hypothèse; nous voulions être en mesure de prévoir comment une population restreinte, après l'enlèvement de tous les sujets, et qui aurait une structure différente par classe de taille, serait distribuée. En comparant ces prévisions à la distribution obtenue deux ans après l'enlèvement des sujets, nous avons constaté que l'hypothèse de la compétition pour une position relative permet des prévisions beaucoup plus justes que l'autre.

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During the summer, Arctic grayling (*Thymallus arcticus*) in interior Alaska's mountain streams get bigger as you go upstream. In larger streams this size gradient can be striking, with the modal length shifting from less than 200 mm in the lower reaches to over 350 mm in the headwaters. Minimum, maximum, and mean lengths of fish within a reach also increase markedly with the progression upstream (Fig. 1). The size gradient is distinct from both spawning and overwintering distributions. It is established soon after spring spawning, when fish may migrate tens of kilometers from spawning areas to their summer feeding areas, and breaks down again in the fall, as fish move to overwintering areas. These whole-stream size gradients are well documented, but only in unpublished reports by the Alaska Department of Fish and Game (see Armstrong 1986 for review).

There are few data available on whole-stream size gradients of stream salmonids, and the subject has apparently never been the main topic of an investigation. Nonetheless, the Arctic grayling's distribution pattern appears to be unusual, because on the rare occasions that the subject of size gradients is explicitly discussed, there is a consensus that fish usually get smaller upstream, salmonids and non-salmonids alike (Brown 1975; Patrick 1975; Milner et al. 1978; Smith 1981; Schlosser 1982; Anderson 1985; Ford and Mercer 1986). The only example of an increase in size with the progression upstream that we could find for any

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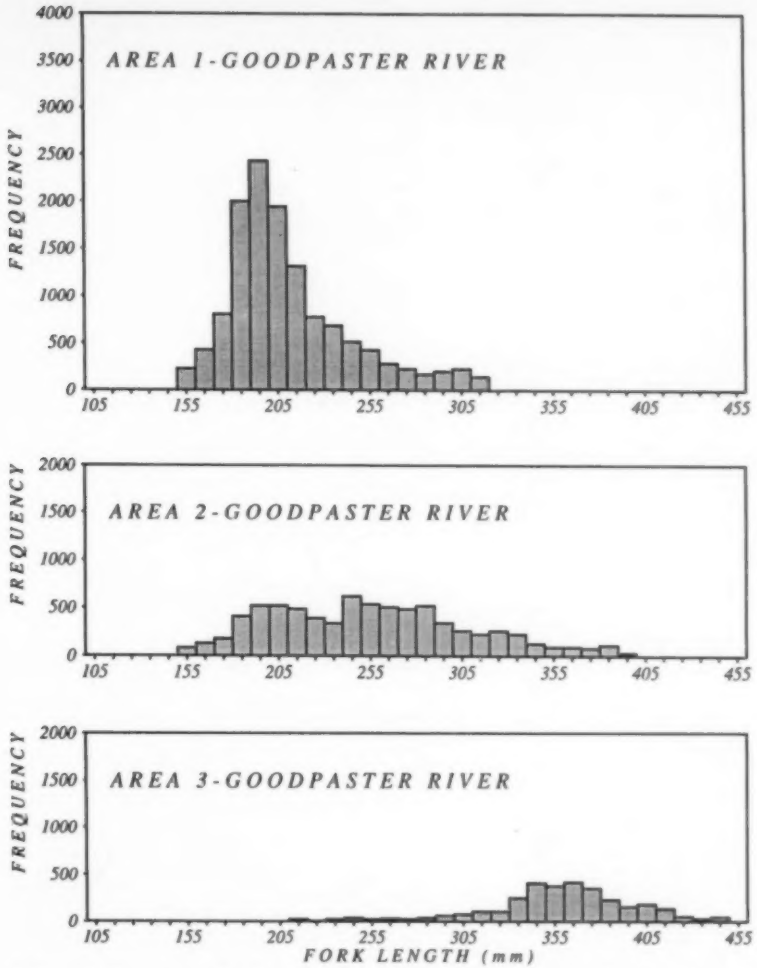

FIG. 1. Length–frequency distribution of Arctic grayling in the Goodpaster River, Yukon Drainage, interior Alaska, showing the increase in fish size with progression upstream. Area 1 includes the lower 53 km of the river, Area 2 the middle 45 km, and Area 3 the upper 87 km (from Tack (1974) with permission).

species of fish is Goto's (1989) study of the river sculpin (*Cottus hangiongensis*).

Since so little attention has been paid to whole-stream size gradients it is not surprising that there is no coherent body of theory to explain how or why they are produced. Authors of studies on nonsalmonid fishes usually hypothesize that growth, mortality, or life history differences between subpopulations of fish in upstream and downstream reaches are responsible for the size gradients (Smith 1981; Anderson 1985; Goto 1989). Ford and Mercer (1986) provide a behavioral explanation, suggesting that American eels (*Anguilla rostrata*) decrease in size with the progression up tidal marsh creeks because big eels exclude smaller ones from preferred territories in the larger creeks. None of these authors mention a third process which could produce size gradients, size-dependent habitat preference. If small fish prefer positions in upstream areas and larger fish prefer positions further downstream (or vice versa) then a size gradient would be produced.

In this paper we describe a removal experiment designed to discriminate between the two hypotheses that we consider the most likely explanations for size gradients of Arctic grayling. We will call these the "competition for ranked positions" hypothesis and the "size-dependent habitat preference" hypothesis respectively. We rejected a third possibility, that growth, mortality, or life history differences between subpopulations of fish in upstream and downstream reaches are responsible, because size gradients of Arctic grayling involve segregation of a single population. This segregation is demonstrated in Fig. 1, which shows that almost all the immature fish in the population are in the lower river, while almost all the mature fish are in the upper river (Arctic grayling in interior Alaska mature at about 5–7 years of age and 270–300 mm fork length).

Under the competition for ranked positions hypothesis, all sizes of fish rank the desirability of available feeding positions along the length of the stream in the same way. Competition then sorts fish so that the biggest fish occupies

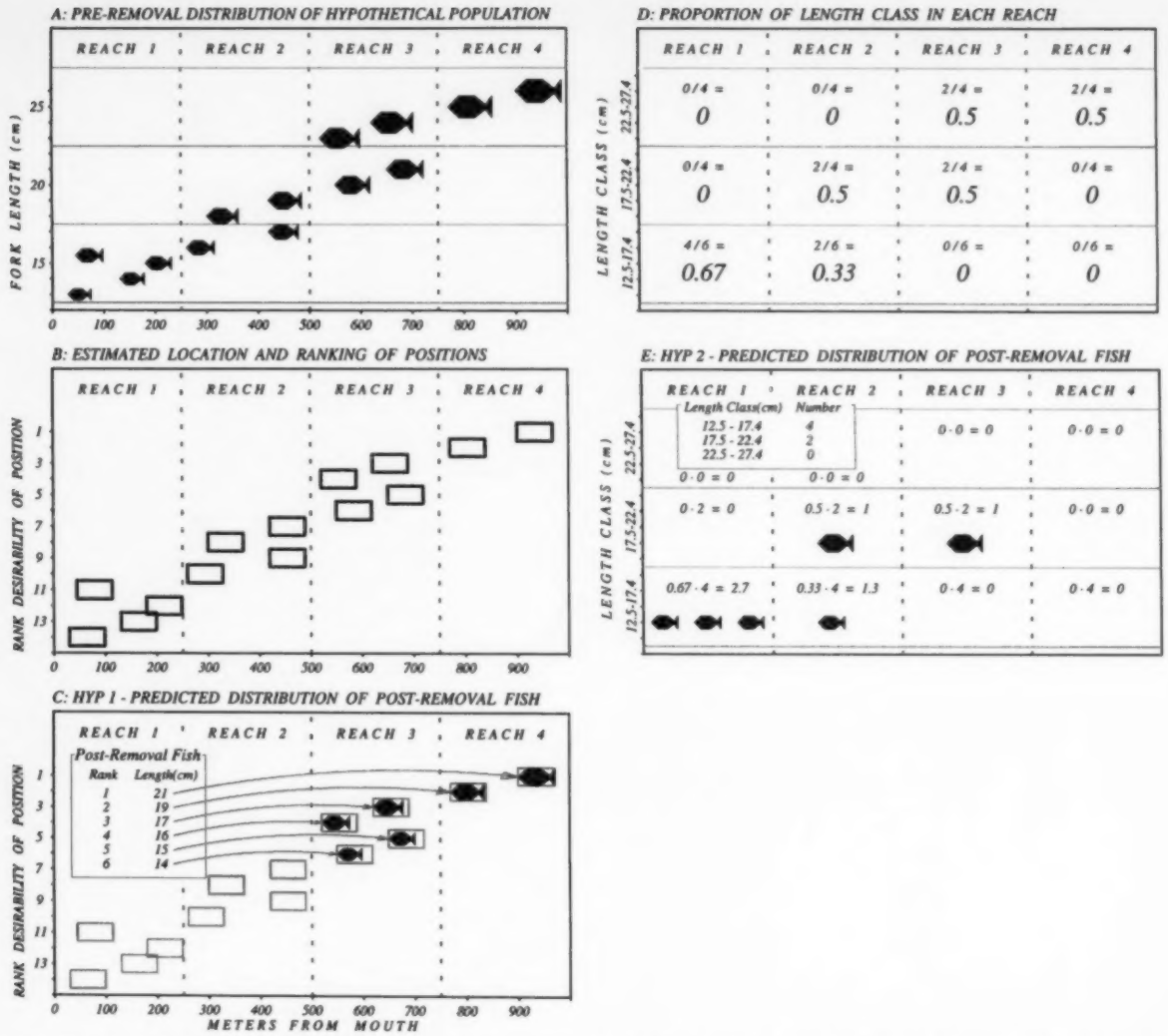

FIG. 2. Hypothetical removal experiment showing how the preremoval distribution pattern is used to predict the postremoval distribution pattern under both the competition for ranked positions hypothesis and the size-dependent habitat preference hypothesis. (A) Preremoval distribution pattern of a population of 14 fish distributed along a stream 1000 meters in length and divided into four reaches. (B) Estimated location and rank desirability of the 14 positions occupied by the preremoval distribution pattern. The rank desirability of each position is the same as the rank length of the fish that occupied it. (C) The predicted distribution pattern of a postremoval population of six fish is made under the competition for ranked positions hypothesis. The largest fish is expected to occupy the No. 1 ranked position, successively smaller fish are expected to occupy successively lower ranked positions. (D) The proportion of each length class in each of the four reaches is calculated from the distribution in panel A (5 cm length classes in this case). These proportions are used as an estimate of the preference each length class has for each reach. (E) The predicted distribution patterns of the same postremoval population of fish used in panel C is made under the size-dependent habitat preference hypothesis. Each length class is allocated to the four reaches according to the same proportions observed for the preremoval population.

the most desirable position and progressively smaller fish occupy progressively less desirable positions. This process is thought to be responsible for within-pool distribution patterns of some salmonids, including Arctic grayling (Newman 1956; Fausch 1984; Hughes and Dill 1990; Hughes 1992a, b). The competition for ranked positions hypothesis assumes that the process responsible for these within-pool distribution patterns also produces whole-stream size gradients. To extend the within-pool explanation to the whole-stream distribution we assume that the desirability of positions tends to increase with the progression upstream. We also assume that competition can distribute fish among positions distributed along the whole stream, a situation involving much larger distances, and many more fish, than would be the case a within a single pool.

Under the size-dependent habitat preference hypothesis a fish's habitat preference changes as it grows. Small fish have a preference for downstream reaches and, as they grow,

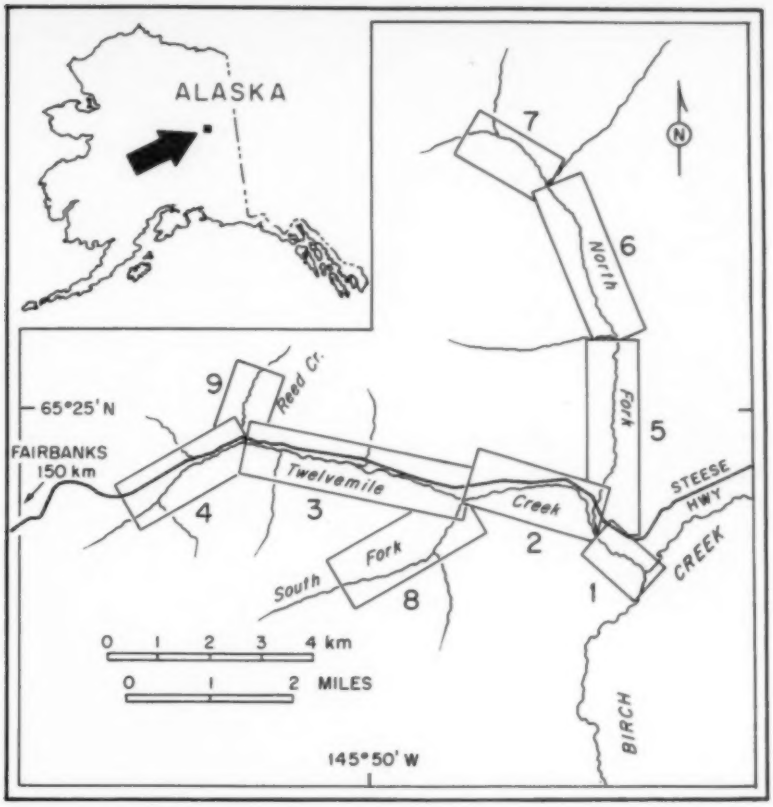

FIG. 3. The Twelvemile Creek drainage, showing its division into nine reaches. Reach boundaries were placed at confluences with major tributaries so that discharge along the length of each reach was roughly constant.

develop a preference for upstream reaches. This hypothesis assumes that competition for feeding positions does not prevent a fish from occupying a reach (although it could influence the position of the fish within that reach). This kind of ontogenetic shift in habitat preference is widespread among salmonids: for example, the shift from freshwater juvenile habitats to marine habitats by anadromous species (Gross 1987), and the shift into faster, deeper water by 0^+ juveniles in streams (Everest and Chapman 1972).

These two hypotheses make very different predictions of how the distribution pattern of Arctic grayling will change if a group of large fish are removed from the headwater reaches. The competition for ranked positions hypothesis predicts that small fish will move into the newly vacated positions, leaving their old positions in downstream reaches empty. The size-dependent habitat preference hypothesis predicts no such change in the distribution pattern. In this paper we describe such a removal experiment. In the first year of the experiment we collected data on the distribution of a population of Arctic grayling in a small stream and simultaneously removed a group of the largest fish. Two years later we again collected data on the distribution of the population, which now had considerably fewer fish and a different length-frequency distribution. We then compared this postremoval distribution pattern with the patterns predicted by the two hypotheses. Figure 2 gives a detailed explanation of the methods used to make these predictions.

Methods

Study Area

Twelvemile Creek is a small mountain stream in interior Alaska (Fig. 3), it is a tributary of Birch Creek, which is in turn a tributary of the Yukon River, and similar to the headwater reaches of most larger rivers in this part of Alaska. The climate is subarctic continental, with long cold winters and short summers, the mean annual temperature is -5.5°C and the annual precipitation about 288 mm·yr^{-1}. Twelvemile Creek starts to freeze up in September and is ice-covered from October through May. Breakup, in late May and early June, is a period of high flow, with the water colored by dissolved organic matter and suspended particulate matter. From mid-June through August the stream generally runs low and clear except after summer rainstorms. Low water discharge during the summer months varies from about 0.03 m^3·s^{-1} at the upstream limit of Arctic grayling distribution to about 0.6 m^3·s^{-1} just before its confluence with Birch Creek.

Twelvemile Creek contains a small isolated population of Arctic grayling because Birch Creek has been heavily

TABLE 1. Abundance and mean fork length of Arctic grayling in the 106 pools of reaches 1–4 of Twelvemile Creek in 1986: Showing length of reach, number of pools in each reach, mean length and range of lengths of pools, number of fish captured in each reach, and mean fork length of fish ±95% CI.

Reach	Reach length (km)	Number of pools	Length of pools (mean and range; m)	Number of fish captured	Mean fork length (±95%CI; mm)
1	0.952	19	32 (4–57)	156	174 ± 0.6
2	3.314	32	20 (4–58)	124	200 ± 0.9
3	5.074	39	13 (2–29)	95	236 ± 1.4
4	3.029	16	8 (2–12)	17	281 ± 2.0

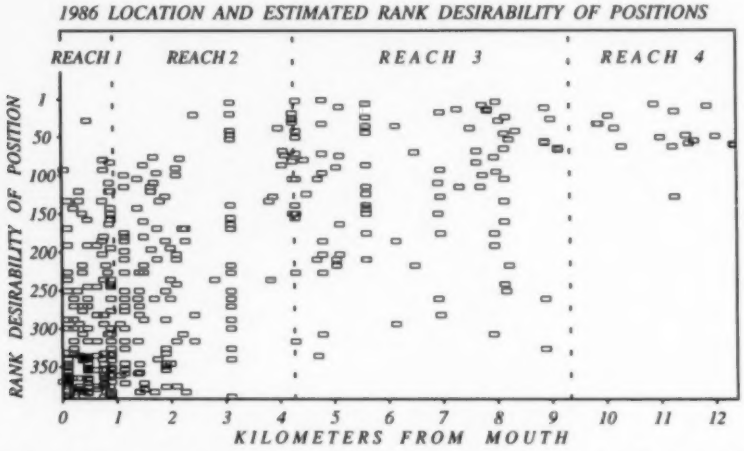

FIG. 4. Rank fork length of the 392 fish inhabiting the pools in reaches 1–4 of Twelvemile Creek during 1986. The largest fish is given rank No. 1 and successively smaller fish are given successively larger ranks. The vertical lines show the reach boundaries.

polluted by placer gold mining since about 1975, and Arctic grayling avoid the high turbidity this creates (McLeay et al. 1987; Reynolds et al. 1989). From mid-June to early September this population exhibits the size gradient typical of much larger systems. In September most fish larger than about 250 mm migrate some tens of kilometers downstream to overwinter in Birch Creek, while smaller fish actually migrate upstream to overwinter in a short section at the upper end of reach 3, where there is groundwater upwelling. In late May and early June, adult fish return to spawn in the lower part of Twelvemile Creek before moving to headwater reaches to feed for the summer (N.F. Hughes, personal observation).

Fish Removal Experiment

To compare the competition for ranked positions hypothesis and the size-dependent habitat preference hypothesis we conducted a big fish removal experiment in the summers of 1986 and 1988. The purpose of this experiment was to change the length distribution and reduce fish abundance in such a way that the two hypotheses made different predictions of how members of the postremoval population should distribute themselves.

In 1986 our survey encompassed the entire drainage (reaches 1–9), from its confluence with Birch Creek to the upstream limit of fish distribution; a total of slightly over 30 km. This complete coverage was necessary because we wanted to remove all the large fish that might move into the more limited area we surveyed postremoval. In the 1986 survey we recorded the fork length of each fish that we caught, and the location of its capture, concurrently removing almost all fish over 260 mm fork length, and 50 fish between 200 and 260 mm fork length. In total, we removed 18% of the total number of fish captured during the survey, or 214 fish out of 1209. We fished each pool and riffle separately, first isolating the section to be fished with blocknets and then making repeated passes through the section with a Smith Root model VII backpack electrofishing unit[3], until we had made two consecutive passes without catching any fish. We assessed the efficiency of this method in capturing fish over 15 cm fork length by releasing marked fish into sections before we electrofished them. Our efficiency in pools was very high (98%; 95% CI 88–100%); our efficiency in riffles was a little lower (85%; 95% CI 69–94%). In this paper our attention focuses on the distribution and abundance of fish in pools, and we treat the electrofishing data as a census, since the differences in the predictions made by our two hypotheses are much larger than the possible sampling error.

In 1988 we used the same methods to determine the postremoval distribution pattern of fish in all 106 pools in reaches 1–4. Our predictions of postremoval distribution patterns apply only to the pools in these four reaches.

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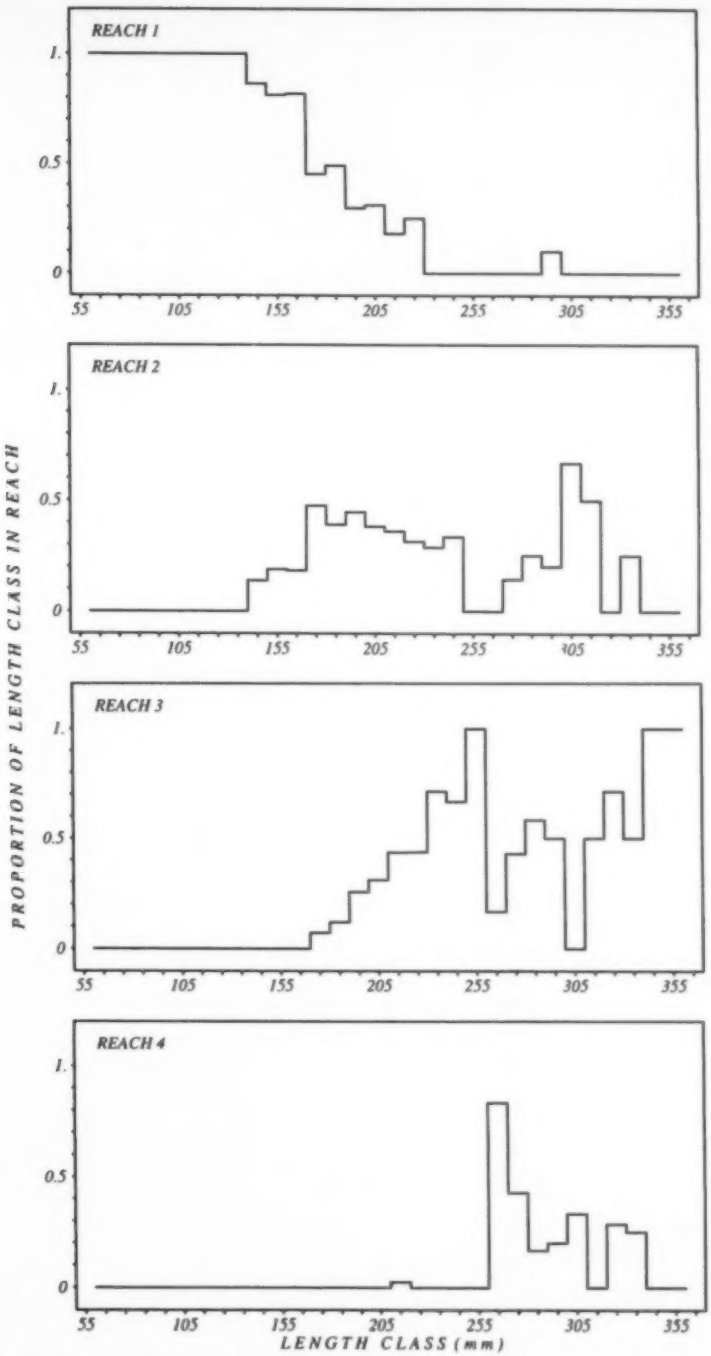

FIG. 5. Proportion of each 1 cm length class found in each of the four reaches of Twelvemile Creek during 1986, pool fish only. Note that there were no fish smaller than 12 cm caught in 1986 and that the allocation of fish of <12 cm fork length was estimated at 1.0 for reach 1 and 0.0 for reaches 2, 3, and 4, based on the fact that 100% of fish in the 12 and 13 cm length groups inhabited reach 1 during 1986. This extrapolation was necessary because some fish <12 cm were caught during 1988.

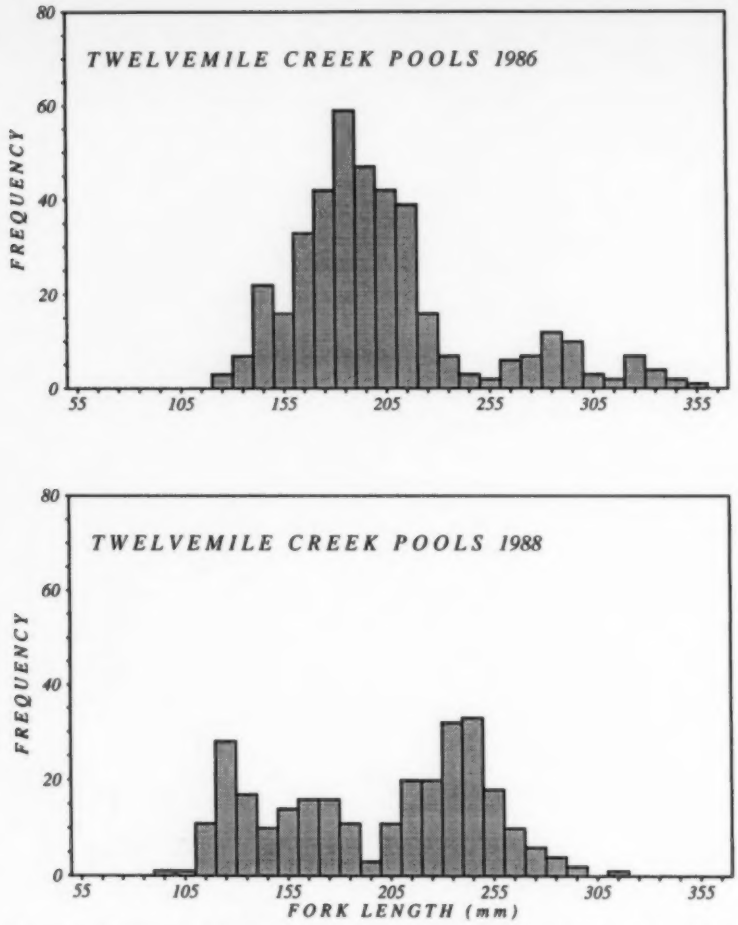

FIG. 6. Length-frequency distribution of Arctic grayling inhabiting pools in reaches 1–4 of Twelvemile Creek during 1986 (392 fish) and 1988 (285 fish).

TABLE 2. Abundance of Arctic grayling occupying pools in reaches 1–4 of Twelvemile Creek in 1986 and 1988, the observed abundance in 1988 as a proportion of observed abundance in 1986, and predicted abundances for 1988 made by the "competition for ranked positions" hypothesis and the "size-dependent habitat preference" hypothesis. The error in the number of fish predicted is shown in parentheses.

	Observed abundance			Predicted abundance, 1988	
Reach	1986	1988	1988 Ab ÷ 1986 Ab	Competition for ranked positions hypothesis	Size-dependent habitat preference hypothesis
1	156	80	0.51	76 (−4)	117 (+37)
2	124	99	0.80	99 (0)	61 (−38)
3	95	87	0.92	93 (+6)	95 (+8)
4	17	19	1.12	17 (−2)	12 (−7)

Results

The preremoval survey of fish distribution revealed the marked increase in fish size with the progression upstream that is typical of much larger systems (Table 1). Data from the pools of reaches 1–4 were used to make the templates with which to predict the postremoval distribution pattern under each of the two hypotheses (Figs. 4, 5).

In 1988 there were only 285 fish in the pools of reaches 1–4, compared with 392 in 1986, and the length-frequency distributions for the two years were markedly different (Fig. 6). These changes were distinctive and allowed us to make a satisfactory comparison of the two hypotheses.

The competition for ranked positions hypothesis made markedly better predictions of fish abundance in each of the four reaches than did the size-dependent habitat preference

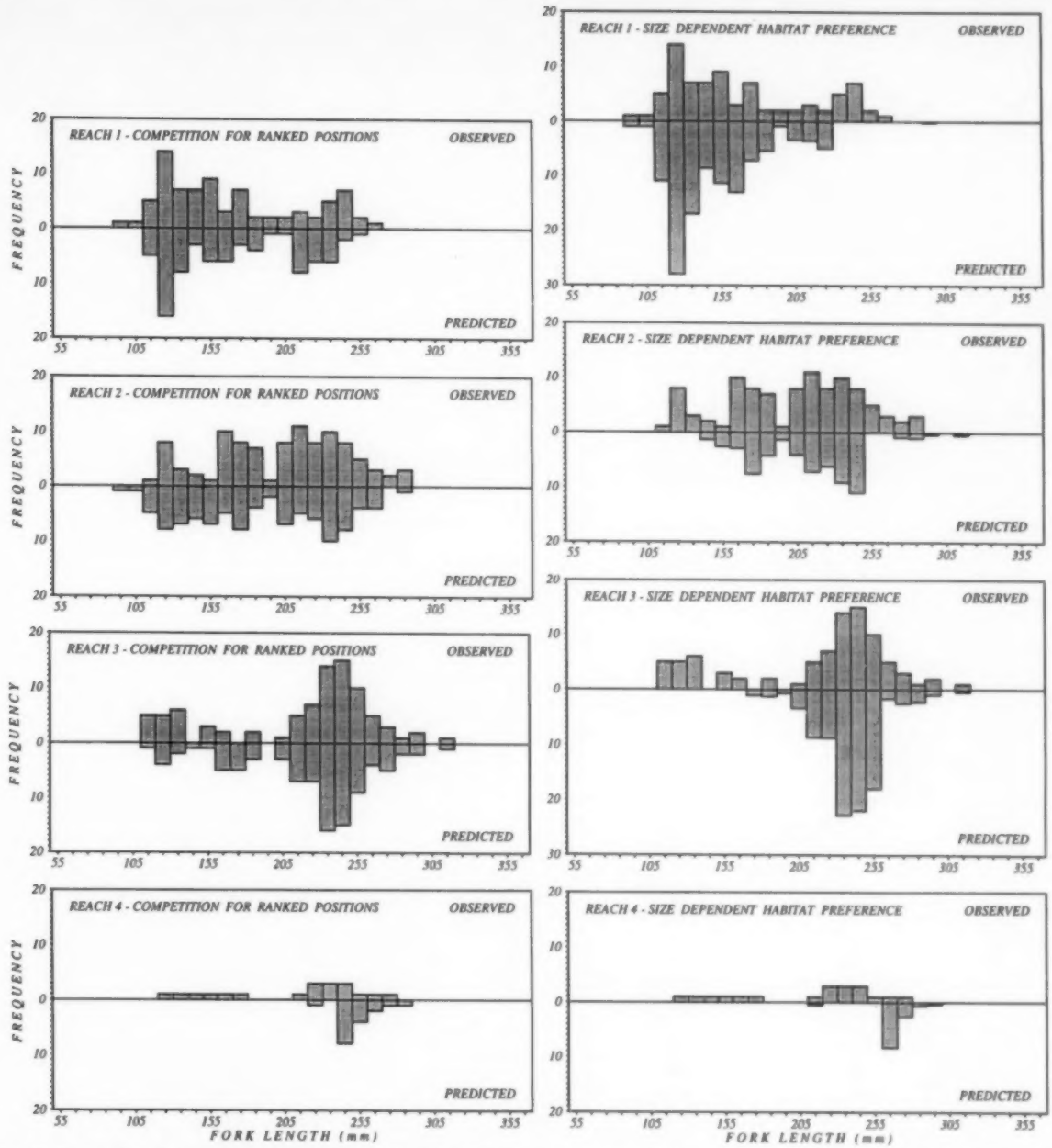

FIG. 7. Comparison between predicted and observed length-frequency distributions of fish inhabiting the pools of reaches 1–4 of Twelvemile Creek during 1988. Left panels: competition for ranked positions hypothesis. Right panels: size-dependent habitat preference hypothesis.

hypothesis (Table 2). Since the observed abundances are census figures this difference is significant. The predictions of the competition for ranked positions hypothesis deviated from observed abundance by 0–6 fish, while the predictions of the size-dependent habitat preference hypothesis deviated by between 7 and 38 fish. The competition for ranked positions hypothesis left only 2% as much unexplained variance as the size-dependent habitat preference hypothesis.

The abundance of fish in reach 1 during 1988 was considerably lower than in 1986, but further upstream the abundance of fish in 1988 approached that in 1986, and matched it in reach 4 (Table 2). Almost all the fish removed in 1986 were taken from reaches 3 and 4, and yet the effects of a reduction in numbers were most evident in reaches 1 and 2. This observation fitted the competition for ranked positions hypothesis well, because when fish abundance decreases, low-ranking positions in downstream reaches should be left

vacant by fish moving upstream to occupy high-ranking positions in upstream reaches.

The competition for ranked positions hypothesis also made substantially better predictions of the length-frequency distribution of fish in each of the four reaches (Fig. 7). The distributions predicted by the competition for ranked positions hypothesis mirror the observed distributions quite closely, both in the size range of fish and in the abundance of each length class. In contrast, the size-dependent habitat preference hypothesis often predicts a truncated size range compared with the observed size range, and its predictions of abundance in each length class are often considerably worse. The competition for ranked positions hypothesis leaves only 38% as much unexplained variance in the assortment of size classes into the four reaches as the size-dependent habitat preference hypothesis.

Discussion

The fish removal experiment leads us to reject the size-dependent habitat preference hypothesis in favor of the competition for ranked positions hypothesis as an explanation for the size gradient of Arctic grayling in Twelvemile Creek. The close match between the predictions of the competition for ranked positions hypothesis and the observed distribution of fish in 1988 gives us additional confidence that the whole-stream size gradient is the result of large fish excluding smaller ones from desirable positions in the upper reaches of streams.

If this conclusion is correct, then the same process responsible for producing patterns of Arctic grayling distribution within a single pool (Hughes 1992a) is also responsible for distribution patterns on a much larger scale, i.e., the size segregation of a population of fish over the length of an entire stream. But can the conclusions arrived at for this small stream, only about twelve kilometers long, be applied to the size gradients in larger systems, such as the Goodpaster River, which are more than ten times as long and contain much larger populations of Arctic grayling? It could be that competition for ranked positions can produce size gradients over relatively short distances that span only a small range of stream habitats, but that size-dependent habitat preference becomes the predominant process in large streams, where the habitats in the upper and lower river are very different.

Although the results of the removal experiment that we report here strongly favor the competition for ranked positions hypothesis, we still think that size-dependent habitat preference may be important in larger streams and that more study is needed to determine whether this is likely. One way to do this would be to conduct a large-scale big fish removal experiment. A second, more practical (though less decisive) approach, would be to develop models that predict which habitats small and large fish *should* prefer, in order to maximize their fitness, or some proxy for fitness such as growth rate. If such models predicted that all sizes of fish would do better in the headwaters, this would favor the competition for ranked positions explanation, since we would expect all fish to compete for positions in the upper river. If, on the other hand, such models predicted that small fish would do better in the lower river, and larger fish would do better in the upper river, then this would favor the size-dependent habitat preference explanation. Ideally, such models would not only explain why Arctic grayling get larger as you go upstream, but also give insight into why this pattern seems to be exceptional, with most species of stream salmonid getting smaller as you go upstream.

Currently, no models explaining the functional basis of stream salmonid size gradients have been developed, but existing data and theory suggest that simple foraging or bioenergetic models may predict size gradients. This is because almost every ecological characteristic of a stream changes along its course (Vannote et al. 1980; Naiman et al. 1987), including those characteristics that influence a fish's energy intake and physiological processes, such as water temperature and invertebrate drift abundance (Walker and Lawson 1977; LaPerriere 1981, 1983).

It is possible that models which include the tradeoffs animals make between rates of energy intake and exposure to predation risk will succeed in explaining size gradients in cases where simpler foraging and bioenergetic models fail. The incorporation of predation risk into foraging models allows better predictions of animal behavior and distribution patterns in many situations (Werner et al. 1983; Power 1984, 1987; Wilzbach 1985; Gilliam and Fraser 1987; Dill 1987; Lima and Dill 1990). However, application of these ideas to whole-stream size gradients of stream fish is difficult because there is no consensus on how mortality risk varies along the course of a stream (see the conflicting interpretations in Smith 1981 and Anderson 1985). It is likely that fish predators will be larger, more numerous, and more effective in the deeper water provided by larger streams (Power 1987; Schlosser 1987), while avian and terrestrial predators may be more effective in small shallow streams (Power 1987; Heggenes and Borgstrom 1988).

If these ideas are true, then it seems unlikely that predation risk is the driving force producing size gradients of Arctic grayling, because smaller fish live in areas that have the greatest abundance of predatory fish (to which small fish are particularly vulnerable) and large fish in areas that probably have the greatest exposure to terrestrial and avian predators (to which large fish are particularly vulnerable). Thus the increase in fish size with progression upstream seems to be exactly opposite to the pattern that would be produced if fish moved into the parts of the stream that minimized their predation risk.

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References

ANDERSON, C.S. 1985. The structure of sculpin populations along a stream size gradient. Environ. Biol. Fishes, 13: 93–102.

ARMSTRONG, R.H. 1986. A review of Arctic grayling studies in Alaska, 1952–1982. Biol. Pap. Univ. Alsk. 23: 3–17.

BROWN, V.M. 1975. Fishes. pp. 199–229. *In* B.A. Whitton [ed.] River ecology. Blackwell Scientific, Oxford.

DILL, L.M. 1987. Animal decision making and its ecological consequences: the future of aquatic ecology and behaviour. Can. J. Zool. 65: 803–811.

EVEREST, F.E., AND D.W. CHAPMAN. 1972. Habitat selection and spatial interaction by juvenile chinook salmon and steelhead trout in two Idaho streams. J. Fish. Res. Board Can. 29: 91–100.

FAUSCH, K.D. 1984. Profitable stream positions for salmonids: relating specific growth rate to net energy gain. Can. J. Zool. 62: 441–451.

FORD, T.E. AND E. MERCER. 1986. Density, size distribution and home range of American eels, *Anguilla rostrata*, in a Massachusetts salt marsh. Environ. Biol. Fishes, 17: 309–314.

GILLIAM, J.F. AND D.F. FRASER. 1987. Habitat selection under predation hazard: a model and test with steam dwelling minnows. Ecology, 68: 1856–1862.

GOTO, A. 1989. Growth differences in males of the river-sculpin *Cottus hangiongensis* along a river course, a correlate of life-history variation. Environ. Biol. Fishes, 24: 241–249.

GROSS, M.R. 1987. Evolution of diadromy in fishes. Am. Fish. Soc. Symp. 1: 14–25.

HEGGENES, J. AND R. BORGSTROM. 1988. Effect of mink, *Mustela vison* Schreber, predation on cohorts of juvenile Atlantic salmon, *Salmo salar* L., and brown trout, *S. trutta* L., in three small streams. J. Fish. Biol. 33: 885–894.

HUGHES, N.F., AND L.M. DILL. 1990. Position choice by drift-feeding salmonids: model and test for Arctic grayling (*Thymallus arcticus*) in subarctic mountain streams, interior Alaska. Can. J. Fish. Aquat. Sci. 47: 2039–2048.

HUGHES, N.F. 1992a. Ranking of feeding positions by drift-feeding Arctic grayling *Thymallus arcticus* in dominance hierarchies. Can. J. Fish. Aquat. Sci. 49: 1994–1998.

HUGHES, N.F. 1992b. Selection of positions by drift-feeding salmonids in dominance hierarchies: model and test for Arctic grayling *Thymallus arcticus* in subarctic mountain steams, interior Alaska. Can. J. Fish. Aquat. Sci. 49: 1999–2008.

LAPERRIERE, J.D. 1981. Chemical and physical influences on invertebrate drift in subarctic Alaskan streams. Ph.D. thesis. Iowa State University, Ames, IA. 64 p.

LAPERRIERE, J.D. 1983. Alkalinity, discharge, average velocity, and invertebrate drift concentration in subarctic Alaskan streams. Freshwater Ecol. 2: 141–151.

LIMA, S.L. AND L.M. DILL. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68: 619–640.

MCLEAY, D.J., I.K. BIRTWELL, G.F. HARTMAN, AND G.L. ENNIS. 1987. Responses of Arctic grayling (*Thymallus arcticus*) to acute and prolonged exposure to Yukon placer mining sediment. Can. J. Fish. Aquat. Sci. 44: 658–673.

MILNER, N.J., A.S. GEE, AND R.J. HEMSWORTH. 1978. The production of brown trout, *Salmo trutta*, in tributaries of the Upper Wye, Wales. J. Fish Biol. 13: 599–612.

NAIMAN, R.J., J.M. MELILLO, M.A. LOCK, T.E. FORD, AND S.R. REICE. 1987. Longitudinal patterns of ecosystems processes and community structure in a subarctic river continuum. Ecology, 68: 1139–1156.

NEWMAN, M.A. 1956. Social behavior and interspecific competition in two trout species. Physiol. Zool. 29: 64–81.

PATRICK, R. 1975. Structure of stream communities. pp. 445–459. *In* M.L. Cody and J.M. Diamond [eds.] Ecology and evolution of communities. The Belknap Press of Harvard University Press, Cambridge, Mass.

POWER, M.E. 1984. Depth distribution of armored catfish: predator-induced resource avoidance? Ecology, 65: 523–528.

POWER, M.E. 1987. Predator avoidance by grazing fishes in temperate and tropical streams: importance of stream depth and prey size. pp. 333–351. *In* W.C. Kerfoot and A. Sih [eds.] Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover and London.

REYNOLDS, J.B., R.C. SIMMONS, AND A.R. BURKHOLDER. 1989. Effects of placer mining discharge on health and food of Arctic grayling. Water Resour. Bull. 25: 625–635.

SCHLOSSER, I.J. 1982. Fish community structure and function along two habitat gradients in a headwater stream. Ecol. Monogr. 52: 395–414.

SCHLOSSER, I.J. 1987. The role of predation in age- and size-related habitat use by stream fishes. Ecology, 68: 651–659.

SMITH, G.R. 1981. Effects of habitat size on species richness and adult body sizes of desert fishes. pp. 125–171. *In* R.J. Naiman and D.L. Soltz [eds.] Fishes in North American deserts. John Wiley and Sons, New York, NY.

TACK, S.L. 1974. Distribution, abundance, and natural history of Arctic grayling in the Tanana River drainage. Alaska Department of Fish and Game, Federal Aid in Fish Restoration, Annual Performance Report, 1973–1974. Project F-9-6, 15(R-1). 52 pp.

VANNOTE, R.L., G.W. MINSHALL, K.W. CUMMINS, J.R. SEDELL, AND C.E. CUSHING. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37: 130–137.

WALKER, J.H., AND J.D. LAWSON. 1977. Natural steam temperature variation in a catchment. Water Res. 11: 373–377.

WERNER, E.E., J.F. GILLIAM, D.J. HALL, AND G.M. MITTLEBACH. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology, 64: 1540–1548.

WILZBACH, M.A. 1985. Relative roles of food abundance and cover in determining the habitat distribution of stream-dwelling cutthroat trout (*Salmo clarki*). Can. J. Fish. Aquat. Sci. 42: 1668–1672.

Survival and Growth of Atlantic Salmon (*Salmo salar*) Fry Stocked at Varying Densities in the White River, Vermont

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Whalen, K.G., and G.W. LaBar. 1994. Survival and growth of Atlantic salmon (*Salmo salar*) fry stocked at varying densities in the White River, Vermont. Can. J. Fish. Aquat. Sci. 51: 2164–2169.

Survival and growth of unfed Atlantic salmon (*Salmo salar*) fry stocked at varying densities (12, 25, 50, and 75 fry·100 m^{-2}) was evaluated in the White River, a tributary of the Connecticut River in Vermont, using a randomized complete block design. Fry to age-0 parr survival was inversely related to fry stocking density and, in most sampling sections, no significant increases in age-0 parr density were attained by stocking at rates ≥50 fry·100 m^{-2}. Most age-0 parr ranged in length between 65 and 80 mm; no significant differences in mean age-0 parr length were detected among stocking densities. Greatest age-0 parr production efficiency may be attained by stocking at densities <50 fry·100 m^{-2}. However, the density of age-0 parr produced from stocking at 12 fry·100 m^{-2} may be insufficient to achieve carrying capacity of age-1 parr.

La survie et la croissance d'alevins du saumon atlantique (*Salmo salar*) non alimentés et introduits en concentrations variables (12, 25, 50 et 75 alevins·100 m^{-2}) dans la rivière White, un tributaire de la rivière Connecticut au Vermont, ont été évaluées au moyen d'un plan expérimental en blocs aléatoires complets. La survie des alevins jusqu'à l'âge des tacons de moins d'un an variait à l'inverse de la densité d'empoissonnement avec les alevins; dans la plupart des sections d'échantillonnage, il n'y a pas eu d'augmentation significative de la densité des tacons d'âge 0 par suite de l'introduction aux taux ≥50 alevins·100 m^{-2}. La longueur de la plupart des tacons d'âge 0 variait entre 65 et 80 mm; on n'a pas trouvé de différence significative de longueur moyenne chez ces mêmes sujets en fonction de la densité d'empoissonnement. C'est en introduisant à des densités <50 alevins·100 m^{-2} qu'on obtient la plus grande efficacité sur le plan de la production de tacons d'âge 0. Toutefois, il se peut que dans le cas d'un empoissonnement au moyen en concentrations de 12 alevins·100 m^{-2}, la densité des tacons d'âge 0 soit insuffisante pour que soit atteint le seuil pour l'obtention de tacons d'âge 1.

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The effort to restore Atlantic salmon (*Salmo salar*) to the Connecticut River basin has been ongoing since the mid-1960s. The production of smolts through fry stocking is a key element in the restoration of Atlantic salmon to the Connecticut River (Stolte 1982). The White River in Vermont is the largest tributary in the Connecticut River system, containing an estimated 13% of the Atlantic salmon nursery habitat (Stolte 1982). For over a decade, the White River and its tributaries have been stocked with Atlantic salmon fry. Currently, federal (United States Forest Service) and state (Vermont Fish and Wildlife (VTF&W)) agencies involved with the White River basin are stocking at densities between 20 and 50 fry·100 m^{-2}.

Several factors influence the fry stocking rates currently used in the White River basin, but fry availability is a particularly important one. In other areas of the salmon range, quantity or quality of habitat may limit overall production, salmonid fry survival (Mason 1976; Egglishaw and Shackley 1977, 1980; Mortensen 1977; Gee et al. 1978*a*; Elliot 1984; Wentworth and LaBar 1984; Hume and Parkinson 1987; see review in Bley and Moring (1988), and, in some cases, growth (Mason 1976; Egglishaw and Shackley 1977, 1980; Randall 1982; Randall and Paim 1982; Scrivener and Anderson 1984) have been shown to be density dependent. Thus, stocking fry at lower densities, allows more habitat to be stocked and reduces the likelihood that fry mortality will result from density-dependent effects.

The survival of unfed fry in the White River and its tributaries has varied greatly among streams stocked at similar densities (John Claussen, VTF&W, Roxbury, VT 05669, unpublished data). This variation in survival at similar densities has made evaluation of the efficacy of stocking within the range of 20 to 50 fry·100 m^{-2} difficult. The objective of this study was to evaluate first-year survival and growth of unfed Atlantic salmon fry stocked at varying densities (12, 25, 50, and 75 fry·100 m^{-2}) and to determine which stocking rate produced the highest residual density of parr per fry stocked. Stocking densities below and above those currently used were also assessed for purposes of comparison.

Study Area

Bethel-Gilead Brook, a tributary of the Third Branch of the White River, Vermont, (Fig. 1) was chosen for this study because it had suitable, relatively uniform habitat and had been stocked with Altlantic salmon (48 fry·100 m^{-2}) since

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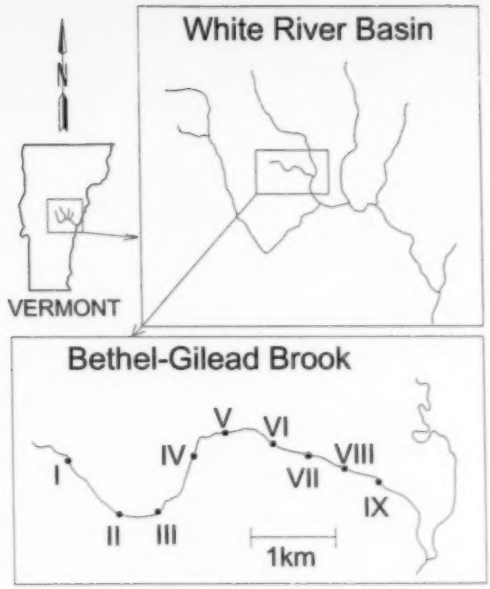

FIG. 1. The White River Basin, Vermont, and Bethel-Gilead Brook including the location of the nine quantitative sampling sites.

1987. Bethel-Gilead Brook arises at an elevation of 487 m and enters the Third Branch of the White River at an elevation of 122 m. The Bethel-Gilead is a second-order stream, with a watershed area of 31 km^2. The wetted width of the brook measured in April 1989 ranged from 6 to 11 m (mean: 8.5 ± 0.4 m (mean ± SD), n = 104). The majority of the watershed is forested, with some agriculture in the middle to lower basin.

Bethel-Gilead Brook contains naturally reproducing populations of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*); catchable-sized brook trout are also stocked in the brook. Trout densities were generally <1.5 fish·100 m^{-2} in stream sections sampled in each year. Other species encountered included blacknose dace (*Rhinichthys atratulus*), longnose dace (*Rhinichthys cataractae*), slimy sculpin, *Cottus cognatus*), white sucker (*Catostomus commersoni*), and creek chub (*Semotilus atromaculatus*).

Bethel-Gilead Brook is a non-natal production stream (Stolte 1982), meaning that it contains suitable rearing habitat for juvenile Atlantic salmon, yet an upstream barrier lower in the watershed precludes passage of adults to the brook. Hence, Atlantic salmon do not reproduce in Bethel-Gilead Brook. Juvenile Atlantic salmon from previous fry stockings were present in the stream.

Methods

Unfed Atlantic salmon fry from the White River National Fish Hatchery (WRNFH) in Bethel, Vermont (progeny of Penobscot River (Maine) sea-run males and domestic Union River (Maine) females), were stocked at three densities each year: 25, 50, and 75 fry·100 m^{-2} in 1989 and 12, 25, and 50 fry·100 m^{-2} in 1990. On two occasions in 1989, the actual stocking density deviated from the planned stocking

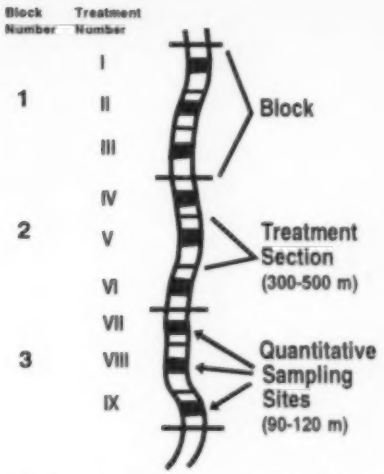

FIG. 2. Schematic of the block, treatment section, and quantitative sampling site arrangement.

TABLE 1. Treatment format. Treatment = fry stocking density (No.·100 m^{-2}).

Block	Section	1989 treatment	1990 treatment
1	I	50	12
	II	31	25
	III	75	50
2	IV	25	50
	V	50	25
	VI	75	12
3	VII	96	50
	VIII	25	12
	IX	50	25

density (Table 1). Hereafter, the target densities of 12, 25, 50, and 75 fry·100 m^{-2} will be referred to as very low, low, medium, and high density treatments, respectively. Fry stocking densities were based on the wetted stream-bed area.

We employed a randomized complete block experimental design to account for confounding variation due to potential differences between upstream and downstream sites. The number of replicates (i.e., blocks) was limited by the length of the stream and the number of treatments. Based on estimates of salmonid fry dispersal (e.g., Egglishaw and Shackley 1982; Wentworth and LaBar 1984; Hume and Parkinson 1987), we determined that for three treatments, a maximum of three blocks could exist. Each treatment section was approximately 500 m in length. In each year, the three treatments were randomly assigned within each of three blocks (Table 1).

Fry were stocked on 17 May 1989 and 9 May 1990. They were evenly distributed along the entire length of the treatment sections, which were contiguous (i.e., there was no unstocked habitat between sections). Within each, a 90- to 150-m length was designated as a quantitative sampling site (Fig. 2). Each quantitative site was separated from the quantitative sampling site of the adjacent treatment by 300–500 m.

We assessed the dominant substrate type and measured water depth and velocity (Marsh-McBirney model 201

TABLE 2. Comparison of key habitat variables measured in the quantitative sampling sites. n = Sample size; 95% confidence limit in parenthesis.

Section	n	Dominant substrate	Mean water depth (cm)	Mean water velocity ($cm \cdot s^{-1}$)	Gradient ($cm \cdot m^{-1}$)
I	42	Rubble	21 (±2)	34 (±6)	1.5
II	57	Gravel	17 (±2)	38 (±6)	1.3
III	54	Gravel/rubble	17 (±2)	27 (±6)	1.0
IV	59	Gravel	16 (±1)	27 (±3)	1.0
V	67	Gravel/rubble	18 (±1)	34 (±6)	0.9
VI	69	Gravel	18 (±2)	27 (±3)	1.2
VII	63	Gravel	16 (±1)	31 (±6)	1.2
VIII	61	Gravel	18 (±2)	31 (±6)	1.5
IX	58	Gravel	19 (±2)	18 (±6)	0.8

TABLE 3. Fry to age-0 survival (S%), age-0 parr population density (No.·100 m^{-2}), mean age-0 parr total length (TL) (mm), and age-1 parr population density by section. Treatment = fry stocking density (No.·100 m^{-2}).

	1989					1990				
		Age-0			Age-1		Age-0			Age-1
Section	Treatment	S%	Density	TL	density	Treatment	S%	Density	TL	density
I	50	16	8	72	1.9	12	24	3	78	3.2
II	31	55	17	72	4.2	25	7	2	74	3.0
III	75	17	13	71	2.4	50	7	4	73	4.9
IV	25	45	11	73	3.3	50	16	8	75	2.9
V	50	29	15	70	3.5	25	18	5	75	3.2
VI	75	39	30	68	3.9	12	44	5	76	4.3
VII	96	16	16	66	1.8	50	13	7	77	4.3
VIII	25	44	11	74	3.0	12	32	4	79	3.9
IX	50	17	9	71	1.5	25	19	5	77	3.0

velocity meter) at nine transects in each quantitative sampling site from 5 to 17 July 1990. Measurements were recorded at 1-m intervals along the length of each transect. For substrate, the type occupying the greatest area within a 1-m^2 frame at each 1-m transect interval was considered dominant. Five substrate categories (Crouse et al. 1981) were used in the assessment. The physical characteristics of the nine quantitative sampling sites were similar (Table 2).

Beginning in mid-August, we determined the population density of age-0 and age-1 parr in each quantitative site via the removal method (Zippin 1958). Fish were collected by electrofishing. Three runs were made between block nets set at each site. With three exceptions over the two sampling years, the probability of capture was ≥0.60, indicating that the electrofishing technique was efficient (White et al. 1982). In 1989, because a large number of age-0 parr was collected at each site, we measured total lengths of a randomly chosen subsample ($n > 30$); all salmon parr collected in 1990 were measured. All parr were anesthetized with MS 222 when measured and redistributed throughout the sampling site following recovery. The number of days between stocking and sampling ranged from 92 to 100 in 1989 and from 96 to 105 in 1990.

Microfish software (Van Deventer and Platts 1985) was used to generate population estimates for each age-class at each site per year. Parr population density (No.·100 m^{-2}) was derived by dividing the parr population estimate by the habitat area sampled. The rate of survival from fry to age-0 parr was calculated by dividing the estimated parr density by the initial fry stocking density. Only survival from fry to age 0 was statistically compared among treatments.

Two-way mixed-model analysis of variance (ANOVA) was used to evaluate treatment differences in fry to age-0 parr survival and in age-0 parr density and length; block was the second factor level in the model. The square root of the survival proportion was transformed to its arc sine before statistical analysis (Zar 1974). Pairwise comparisons were performed using Duncan's Multiple Range Test (DMRT), if the F-test of the treatment ANOVA was significant. Regression analysis was used to examine associations between age-0 and age-1 parr densities and the relationship between age-0 parr census density and length. Ninety-five percent confidence intervals, based on a pooled estimate of variance, are reported apposite to the treatment means. Statistical significance was determined at the $\alpha = 0.05$ level.

Results

Fry to Age-0 Parr Survival and Age-0 Parr Density

Survival from fry to age-0 parr ranged from 16 to 55% in 1989 and from 7 to 44% in 1990 (Table 3, Fig. 3). Although fry survival ranged widely, significant differences (ANOVA; $p < 0.05$) in mean survival among treatments were detected in both years. In 1989, the mean survival of fry stocked at the low density (48 ± 11%) was significantly higher than the mean survival of fry at the medium (21 ± 11%) and high density treatments (24 ± 11%) (DMRT; $p < 0.05$). Survival at the medium and high densities was not

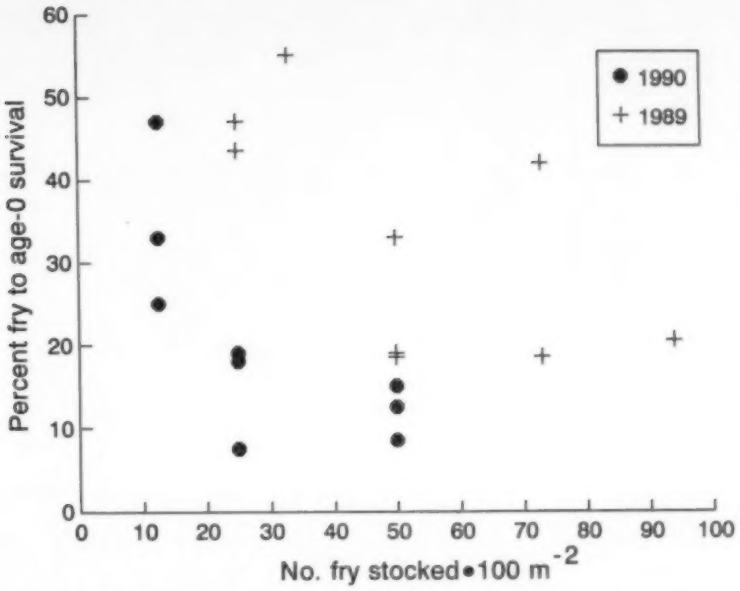

FIG. 3. Percent fry to age-0 survival and fry stocking density (No.·100 m^{-2}); 1989 and 1990.

significantly different. In 1990, the mean survival of fry in the very low density treatment (33 ± 5%) was significantly higher than the mean survival of fry stocked at the low (15 ± 5%) and medium (12 ± 5%) densities (DMRT; $p < 0.05$); the difference in mean survival between the low and medium densities was not significant. The block effect on survival was significant in 1990 (ANOVA; $p < 0.05$), but not in 1989.

Age-0 parr density ranged from 8 to 30 parr·100 m^{-2} in 1989 and from 2 to 8 parr·100 m^{-2} in 1990 (Table 3). In 1989, age-0 parr density was not significantly different among the low, medium, and high treatments, but it was significantly different (ANOVA; $p < 0.05$) among treatments in 1990. The mean age-0 density produced from stocking at 50 fry·100 m^{-2} was significantly higher (DMRT; $p < 0.05$) than the mean age-0 parr density produced from very low and low density fry stocking (Table 3).

Age-1 Parr Density

The stream-wide mean density at age-1 was 2.8 ± 0.8 parr·100 m^{-2} in 1989 and 3.6 ± 0.6 parr·100 m^{-2} in 1990. In 1989, the density of age-0 and age-1 parr observed at each section was moderately positively correlated ($r = 0.59$; $p < 0.10$). No relationship existed between the density of age-0 parr in a section in 1989 and the subsequent density of age-1 parr in that section in 1990.

Age-0 and Age-1 Parr Length

The initial mean length of fry in 1989 was 27 ± 1 mm ($n = 30$) and 26 mm in 1990. (In 1990, fry lengths were estimated from hatchery growth charts; thus no confidence limits are reported.) In 1989 for each treatment, the mean length of age-0 parr was 73 ± 3 mm (low), 71 ± 3 mm (medium), and 68 ± 3 mm (high); and in 1990, 78 ± 2 mm (very low), 75 ± 2 mm (low), and 75 ± 2 mm (medium). In both 1989 and 1990, differences in mean age-0 parr length between treatments were not statistically significant. In 1989, the mean length and density of age-0 parr observed at each section were moderately inversely correlated ($r = -0.58$; $p < 0.10$) (Table 3).

In 1990, the mean length of age-1 parr collected at each section ranged from 117 to 126 mm (121 ± 1 mm).

Discussion

We provide evidence of density-dependent survival in the recruitment of stocked Atlantic salmon fry to yearling parr. Density-dependent survival was also observed with juvenile steelhead (Wentworth and LaBar 1984; Hume and Parkinson 1987), brown trout (Mortensen 1977; Elliot 1984), and Atlantic salmon (Egglishaw and Shackley 1977, 1980; Gee et al. 1978*a*) in other studies. Our results for both age-0 and age-1 parr are consistent with the notion of Symons (1979) that a "ceiling" for Atlantic salmon parr populations exists in streams, where mortality rather than population density increases as fry stocking density increases past carrying capacity. This evidence of density-dependent survival in the recruitment of stocked unfed Atlantic salmon fry is significant because fry stocked in excess of the carrying capacity could theoretically have been placed in other streams where they would have had the opportunity to contribute to the cohort. Minimizing density-dependent mortality in the recruitment of juvenile salmon is essential for maximizing the efficiency of production, which is important for salmonid restoration efforts such as the Connecticut River program where fry availability rather than habitat is limited. Because the specific fry stocking densities we used may not be applicable elsewhere (O'Connell and Bourgeois 1987), recognizing the density at which stocked fry recruitment is affected by density-dependent mortality may be the most important aspect of the analysis of fry stocking densities.

The interaction of population density (i.e., fry stocking density), density-independent mortality, and stream carrying

capacity (Fox and Flowers 1990) determines whether fry stocked at higher densities survive and lead to increased production or die. When the carrying capacity of a reach is high and fry are stocked at higher densities, a substantial increase in age-0 parr production may be achieved, as evidenced by the results from section VI in 1989. However, when age-0 carrying capacity is lower, density-dependent mortality limits increases in age-0 densities expected from stocking fry at higher densities, as evidenced from the results from the remaining medium and high density treatment sections. A high residual density of age-0 parr such as the one observed at section VI is insignificant if it causes a density-dependent response in the recruitment of parr from age-0 to age-1. The residual age-0 density of 30 parr·100 m^{-2} observed at section VI did not lead to a significantly higher density of age-1 parr in 1990 than what was observed at the remaining sections. Mason (1976) observed that such dramatic increases in age-0 parr densities may be nullified by insufficient suitable winter habitat.

Because the number of returning adults is generally proportional to the number of smolts leaving a river (Chadwick et al. 1978), a primary goal of Atlantic salmon restoration is to maximize recruitment throughout each life stage to maximize the number of smolts produced. Thus, while it is important to minimize the density-dependent aspect of fry recruitment to maximize production efficiency, the absolute number of parr produced from fry stocking must also be considered. For example, while the efficiency of parr production (parr produced per fry stocked) in 1990 was greatest at 12 fry·100 m^{-2}, the resulting mean density of 4 age-0 parr·100 m^{-2} may not be sufficient to achieve the age-1 densities of 3 fry·100 m^{-2} that have been observed in the brook.

Juvenile Atlantic salmon survival may range widely from year to year because of yearly differences in density-independent (e.g., temperature) and/or density-dependent (e.g., predation rates) factors controlling the population density (Symons 1979). The factor affecting age-0 parr carrying capacity in 1990 was stream-wide, as the age-0 parr density was lower in 1990 than in 1989 at all sections. We were unable to identify a specific factor responsible for the difference observed in age-0 parr carrying capacity between years. It is known that Atlantic salmon yearlings may negatively affect conspecific underyearlings (Elson 1975; Symons and Heland 1978; Buck and Hay 1984; Kennedy and Strange 1986). Densities of age-1 and age-0 parr, however, were not inversely related in either year. We recognize that our inability to detect an inverse relationship between year-classes does not imply that competitition between them was absent, rather evidence of competition was inconclusive. More research is needed to determine the effect of yearling pressure on the survival of fry stocked at densities between 20 and 50 fry·100 m^{-2}, due to the potential negative effects of inter-year-class, intraspecific competition.

Dispersal of fry and parr from the locus of stocking was a potential confounding factor (Symons 1979) for our stocking density evaluation. However, localized within-treatment movements could have occurred without compromising the experiment. The entire treatment section, including the quantitative sampling site, was stocked at a uniform density; localized emigration and immigration were thus equally probable. Dispersal would have confounded the experiment if fry or parr had moved from one quantitative sampling site to another. The dispersal of anadromous salmonid fry has been shown to be limited (Gee et al. 1978*b*; Egglishaw and Shackley 1982; Wentworth and LaBar 1984; Hume and Parkinson 1987). Thus, for fry up to age-0 of the first growing season we assumed that confounding cross-treatment interference was unlikely, because of the distances between the quantitative sampling sites of adjacent treatments (300–500 m). For older parr, however, that are known to disperse farther from the initial locus of stocking than fry (Hume and Parkinson 1987), the potential for cross-treatment interference was greater. Hence, our statistical analysis of treatment-specific survival was limited to age-0 parr, because we could not assume that age-1 parr collected from a quantitative site were derived from fry stocked in the respective treatment section.

Although other studies involving juvenile salmonids (e.g., Mason 1976; Egglishaw and Shackley 1977, 1980; Randall 1982; Randall and Paim 1982; Scrivener and Anderson 1984) have reported density-dependent growth, age-0 parr length in our study was not significantly different among treatments in either year and was only moderately inversely correlated to the age-0 census density in 1989. Grant and Kramer (1990) noted that studies involving juvenile salmonids that have reported density-dependent survival have not always reported density-dependent growth. We acknowledge that our inability to observe significant differences in mean age-0 parr length among treatments may have been a function of statistical power (Peterman 1990). Practically, the relationship between population density and growth is somewhat tenuous, because growth is highly dependent upon abiotic factors (Egglishaw and Shackley 1977, 1985; Zalewski et al. 1985). Parr were in fact sampled before the completion of the growing season. In addition, timing may have been an important factor affecting the expression of density-dependent growth. For stocked salmonid fry, high mortality rates have been observed early in the growing season (Egglishaw and Shackley 1977; Wentworth and LaBar 1984). The regulation of population density soon after stocking would allow fry initially stocked at higher densities to exist at relatively low densities for much of the growing season. Thus, density-dependent survival could still be manifested, without a concomitant density-dependent growth effect.

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References

Bley, P.W., and J.P. Moring. 1988. Freshwater and ocean survival of Atlantic salmon and steelhead: a synopsis. U.S. Fish Wildl. Serv. Biol. Rep. 88: 22 p.

Buck, R.J.G., and D.W. Hay. 1984. The relation between stock size and progeny of Atlantic salmon (*Salmo salar*) in a Scottish stream. J. Fish Biol. 23: 1–11.

Chadwick, E.M.P., T.R. Porter, and P. Dowington. 1978. Analysis of growth of Atlantic salmon (*Salmo salar*) in a small Newfoundland river. J. Fish. Res. Board Can. 35: 60–68.

Crouse, M.R., C.A. Callahan, K.W. Malueq, and S.E. Dominguez. 1981. Effects of fine sediments on growth of juvenile coho salmon in laboratory streams. Trans. Am. Fish. Soc. 110: 281–286.

EGGLISHAW, H.J., AND P.E. SHACKLEY. 1977. Growth, survival, and production of juvenile salmon and trout in a Scottish stream. J. Fish Biol. 11: 647–672.

EGGLISHAW, H.J., AND P.E. SHACKLEY. 1980. Survival and growth of salmon (*Salmo salar*) planted in a Scottish stream. J. Fish Biol. 16: 565–584.

EGGLISHAW, H.J., AND P.E. SHACKLEY. 1982. Influence of water depth on dispersion of juvenile salmonids, (*Salmo salar*) and (*Salmo trutta*) in a Scottish stream. J. Fish Biol. 21: 141–155.

EGGLISHAW, H.J., AND P.E. SHACKLEY. 1985. Factors governing the production of juvenile salmon in Scottish streams. J. Fish Biol. 27: 27–33.

ELLIOT, J.M. 1984. Numerical changes and population regulation in young migratory trout (*Salmo trutta*) in a Lake District stream, 1966–83. J. Anim. Ecol. 53: 327–350.

ELSON, P.F. 1975. Atlantic salmon rivers, smolt production and optimal spawning: an overview of natural reproduction. Int. Atl. Salmon Found. Spec. Publ. Ser. 6: 99–119.

FOX, M.G., AND D.D. FLOWERS. 1990. Effect of fish density on growth, survival, and food consumption by juvenile walleye in rearing ponds. Trans. Am. Fish. Soc. 119: 112–121.

GEE, A.S., N.J. MILNER, AND R.J. HEMSWORTH. 1978a. The effect of density on mortality in juvenile Atlantic salmon (*Salmo salar*). J. Anim. Ecol. 47: 497–505.

GEE, A.S., N.J. MILNER, AND R.J. HEMSWORTH. 1978b. The production of juvenile Atlantic salmon (*Salmo salar*) in the upper Wye, Wales. J. Anim. Ecol. 47: 497–505.

GRANT, J.W.A., AND D.L. KRAMER. 1990. Territory size hypothesis as a predictor of the upper limit to population density of juvenile salmonids in streams. Can. J. Fish. Aquat. Sci. 47: 1724–1737.

HUME, J.M., AND E.A. PARKINSON. 1987. Effect of stocking density on the survival, growth, and dispersion of steelhead fry (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 44: 271–281.

KENNEDY, G.J.A., AND C.D. STRANGE. 1986. The effects of intra- and interspecific competition on the survival and growth of stocked juvenile Atlantic salmon, (*Salmo salar*) and resident trout, (*Salmo trutta*) in an upland stream. J. Fish Biol. 28: 479–489.

MASON, J.C. 1976. Response of underyearling coho salmon to supplemental feeding in a natural stream. J. Wildl. Manage. 40: 775–788.

MORTENSEN, E. 1977. Population, survival, growth and production of trout (*Salmo trutta*) in a small Danish stream. Oikos, 28: 9–15.

O'CONNELL, M.F., AND C.E. BOURGEOIS. 1987. Atlantic salmon enhancement in the Exploits River, Newfoundland, 1957-1984. N. Am. J. Fish. Manage. 7: 207–214.

PETERMAN, R.M. 1990. The importance of reporting statistical power: the forest decline and acidic deposition example. Ecology, 71: 2024–2027.

RANDALL, R.G. 1982. Emergence, population densities, and growth of salmon and trout fry in two New Brunswick streams. Can. J. Zool. 60: 2239–2244.

RANDALL, R.G., AND U. PAIM. 1982. Growth, biomass, and production of juvenile Atlantic salmon (*Salmo salar* L.) in two Miramichi River, New Brunswick, tributary streams. Can. J. Zool. 60: 1647–1659.

SCRIVENER, J.C., AND B.C. ANDERSON. 1984. Logging impacts and some mechanisms that determine the size of spring and summer populations of coho salmon fry (*Oncorhynchus kisutch*) in Carnation Creek, British Columbia. Can. J. Fish. Aquat. Sci. 41: 1097–1105.

STOLTE, L.W. 1982. A strategic plan for the restoration of Atlantic salmon to the Connecticut River basin. U.S. Fish and Wildlife Service, Laconia, N.H.

SYMONS, P.E.K. 1979. Estimated escapement of Atlantic salmon (*Salmo salar*) for maximum smolt production in rivers of different productivity. J. Fish. Res. Board Can. 36: 132–140.

SYMONS, P.E.K., AND M. HELAND. 1978. Stream habitats and behavioral interactions of underyearling and yearling Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 35: 175–183.

VAN DEVENTER, J.S., AND W.S. PLATTS. 1985. A computer software system for entering, managing, and analyzing fish capture data from streams: user's guide. USDA, For. Serv. Intermountain For. Range Exp. Stn. Res. Note INT-352: 22 p.

WENTWORTH, R.S., AND G.W. LABAR. 1984. First-year survival and growth of steelhead stocked as fry in Lewis Creek, Vermont. N. Am. J. Fish. Manage. 4: 103–110.

WHITE, G.C., D.R. ANDERSON, K.P. BURNHAM, AND D.L. OTIS. 1982. Capture–recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, Los Alamos, N.M. LA-8787-NWEP: 107–108.

ZALEWSKI, M., P. FRANKIEWICZ, AND B. BREWINSKA. 1985. The factors limiting growth and survival of brown trout (*Salmo trutta* m. fario L.) introduced to different types of streams. J. Fish. Biol. 27 (suppl. A.): 59–73.

ZAR, J.H. 1974. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, N.J.

ZIPPIN, C. 1958. The removal method of population estimation. J. Wildl. Manage. 22: 82–90.

Differences in Measurements of Smolt Development Between Wild and Hatchery-Reared Juvenile Coho Salmon (*Oncorhynchus kisutch*) Before and After Saltwater Exposure

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We compared the saltwater tolerance of coho salmon (*Oncorhynchus kisutch*) juveniles that were reared in different environments. The groups examined consisted of fish reared exclusively in the hatchery, a hatchery group transplanted into the upper watershed of the river (colonized), and wild fish from natural spawning broodstock in the river. Although hatchery fish were much larger than their wild or colonized counterparts, they consistently showed a reduced saltwater tolerance as assessed by a much greater perturbation in plasma sodium concentration following transfer to salt water. Within each group there was no relationship between size of the fish and saltwater tolerance. Following transfer to sea water, hatchery fish showed a significant decline in haematocrit and a significant increase in circulating plasma cortisol concentration. Neither of these changes was seen in wild smolts. Hatchery fish possessed fewer chloride cells, and lower specific activities of the enzymes Na^+K^+ATPase and citrate synthase. The weaker osmoregulatory ability of hatchery fish led to a greater mortality following abrupt transfer to 35‰ seawater. We believe that the differences in saltwater tolerance seen among the different groups of fish are due to rearing environment.

Nous avons comparé la tolérance à l'eau salée de juvéniles du saumon coho (*Oncorhynchus kisutch*) qui ont été élevés dans différents environnements. Il s'agissait de sujets élevés exclusivement en pisciculture, de groupes de pisciculture introduits dans la partie amont du bassin versant d'une rivière (sujets colonisateurs) et de poissons à l'état sauvage issus de géniteurs venus se reproduire dans la rivière. Même s'ils étaient beaucoup plus gros que les poissons colonisateurs ou que ceux à l'état sauvage, les poissons d'élevage marquaient régulièrement une tolérance moindre à l'eau de mer, qui se mesurait à une perturbation nettement supérieure de la concentration sodique du plasma après transfert dans l'eau salée. À l'intérieur d'aucun groupe n'a-t-on observé de relation entre la taille du poisson et la tolérance à l'eau salée. Après transfert dans l'eau de mer, l'hématocrite des poissons d'élevage diminuait de manière significative et la concentration circulante de cortisol plasmatique s'accroissait de manière significative. Ni l'un ni l'autre de ces changements n'a été observé chez les saumoneaux à l'état sauvage. Les poissons d'élevage possédaient moins de cellules à chlorures et leurs enzymes Na^+K^+ATPase et citrate synthase avaient une moins grande activité spécifique. L'osmorégulation moins efficace des poissons d'élevage est à l'origine de la plus grande mortalité qui a suivi le transfert brutal à l'eau de mer à 35‰. Nous croyons que les différences observées au niveau de la tolérance à l'eau salée entre les différents groupes sont attribuables à l'environnement dans lequel les poissons ont été élevés.

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Coho salmon (*Oncorhynchus kisutch*) is an economically important species for the commercial and sport fisheries of British Columbia. A large number of enhancement facilities have been established to increase the number of smolts released into streams, with the objective of supplementing the wild fishery. It is important, therefore, to produce a fish with as great a chance of survival as possible. The rearing environment within a hatchery has been shown to have a substantial impact on development and smolting in coho salmon (Schreck et al. 1985; Patino et al. 1986). Smolt-to-adult survival in chinook salmon (*O. tshawytscha*) is greatest for fish that show a high degree of smolt development prior to release from the hatchery (Zaugg and Mahnken 1991).

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Adult returns of salmonids produced within British Columbia hatchery systems, however, have declined over the past decade (Cross et al. 1991). It is not clear what factor is limiting the success of salmonids that are produced within the hatchery system. Poor ocean survival of coho and chinook smolts produced by enhancement facilities has been suggested as a possible explanation for the decline in these salmon stocks (Walters 1988; Wedemeyer et al. 1980).

The migration of smolts into the ocean is timed to coincide with a number of morphological and physiological changes that prepare the fish for transition into salt water. In fresh water, fish must actively take up ions and prevent the influx of water to maintain body ion homeostasis. In seawater, they must actively excrete ions and drink water to compensate for water lost to the environment. The process of smoltification preadapts fish to salt water and minimizes perturbations in plasma ions following transition into salt water.

Typically, transition from fresh water to the marine environment is a time of high mortality in juvenile salmonids (Healey 1982). Fisher and Pearcy (1988) concluded that the success of a year-class was determined early in the summer, soon after most juvenile coho had entered the ocean. It would appear that the development stage of fish that are released from the hatchery is critical for the survival of these animals to adult (Zaugg and Mahnken 1991).

We have conducted experiments to compare differences in saltwater tolerance between hatchery-reared and wild juvenile coho salmon. Quinsam River coho salmon were used for this investigation. The Quinsam River provided a unique opportunity to study the effects of rearing environment on selected aspects of the physiology of coho salmon and to compare wild and hatchery populations of fish derived from a common stock. In addition, a colonization program for hatchery fry released into the upper Quinsam watershed made it possible to compare fish of hatchery origin, reared in a wild environment, with fish that were reared exclusively within a hatchery. Experiments were conducted during May of 1990, 1991, and 1992.

Materials and Methods

Study Site

The Quinsam River is located northwest of the town of Campbell River, Vancouver Island, British Columbia. The river is approximately 40 km long, and drains an extensive network of tributaries and lakes, but only the lower 29 km of the river are accessible to coho salmon. The Quinsam River is the main tributary of the Campbell River and their confluence occurs 3.5 km above the Campbell River estuary (Blackmun et al. 1985). In 1974, a Salmonid Enhancement Program Facility was constructed on the Quinsam River. The hatchery is located approximately 1 km above the confluence with the Campbell River. The hatchery production of coho smolts is greater than 120 000 each year. Production of wild smolts in the Quinsam River is estimated to be greater than 35 000 per year. In addition, a colonization program of hatchery fry transplanted to the upper Quinsam River watershed produces more than 20 000 smolts per year. Consequently, a majority of coho smolts produced in the Quinsam River system are of hatchery origin (D. Ewart, Quinsam River Salmonid Hatchery, Box 467, Campbell River, BC V9W 5C1, personal communication).

TABLE 1. Mean weight and fork length (± SE) of the wild, colonized, and hatchery Quinsam River coho salmon used in the saltwater challenge experiments.

	1990	1991	1992
Wild			
Weight (g)	8.03±0.27	11.77±0.38	9.75±0.57
Length (cm)	9.65±0.11	10.85±0.14	10.26±0.19
n	32	100	42
Colonized			
Weight (g)	16.17±0.94		
Length (cm)	12.28±0.22		
n	33		
Hatchery 1			
Weight (g)	27.34±0.90	21.36±0.53	24.71±0.98
Length (cm)	14.19±0.15	13.18±0.10	13.63±0.16
n	34	106	44
Hatchery 2			
Weight (g)	25.87±1.44	22.92±0.73	26.79±1.10
Length (cm)	13.95±0.22	13.38±0.12	14.06±0.16
n	31	97	40

Fish

Juvenile coho salmon from the Quinsam River were used to examine the effect of rearing environment on saltwater tolerance over 3 yr, 1990–1992. In 1990, four groups were chosen; wild, colonized, hatchery 1, and hatchery 2. Wild smolts were fish that had developed from eggs spawned naturally in the Quinsam River. Colonized fish had been spawned and raised at the Quinsam River Salmon Hatchery for 10 mo and then released as fry, in September, into the upper Quinsam watershed. The fish resided in the upper Quinsam until May of the following year, at which time they migrated downstream. This group was not marked but could be distinguished from the wild smolts on account of their larger size. Earlier marking studies showed that a bimodal size distribution existed for the fish migrating down the Quinsam river (D. Ewart, Quinsam River Salmonid Hatchery, personal communication). Hatchery 1 and hatchery 2 were juveniles that had been reared at the Quinsam River Hatchery in the top (water inflow) and bottom sections (water outflow), respectively, of a three-section earthen channel supplied with groundwater. The water was first passed through rearing containers on the Quinsam upper site and then through a clarifier to remove gross particulate matter. The outflow from the clarifier fed into the top section of the earthen channel. The mean hatchery water temperature was approximately 10°C with a seasonal fluctuation of less than 2°C. In contrast, the temperature of the river water showed a large seasonal variation from a low of less than 2°C in the winter, to a maximum of greater than 15°C prior to release in the spring. In 1991 and 1992, colonized fish were not included in the saltwater challenge (SWC) tests. Mean lengths and weights of fish from all groups used over the three years in this study are presented in Table 1.

Experimental Procedures and Sampling

In May 1990, wild and colonized smolts were caught during their downstream migration and held overnight in the trap at the downstream enumeration fence across the

Quinsam River. The fish were then transferred to aluminum troughs. The two hatchery groups were seined from the top and bottom of the earthen channel. All four groups were held in aluminum troughs for less than a week and starved for 48 h prior to experimentation. To examine hypo-osmoregulatory ability, the fish were transferred into 100-L buckets containing salt water on May 25, 1990. Ten to 12 fish were placed in each bucket. The density of fish in the bucket was maintained below 3.0 $g \cdot L^{-1}$, as recommended by Blackburn and Clarke (1987) for a saltwater challenge. Each bucket that held fish was part of a recirculating saltwater system consisting of three 100-L buckets. Two buckets held the fish and drained into a common reservoir between them where water was aerated and then pumped back into the buckets containing the fish. Several recirculation systems were placed in an aluminum trough and surrounded with flowing fresh water to maintain constant temperature. Despite the cooling system, there were slight increases in water temperature during the day (<3°C), but these temperature changes were similar for all groups. Dissolved oxygen levels did not drop below 8.5 ppm during the day. Approximately one-third of the water was replaced daily to maintain water quality.

In 1990, fish were sampled before exposure (0 d), and after 1 and 7 d in salt water. Salinity of the water during the challenge was 29‰, and the temperature averaged 12.6°C. At designated intervals, fish were killed by a cephalic blow, the caudal peduncle was severed, and blood was collected in heparinized capillary tubes. The tubes were centrifuged for 5 min at 11 500 × *g*, haematocrits were read, and plasma was saved. Approximately 50 mg of gill tissue was removed from each fish and placed in 0.5 mL of ice cold SEI (0.3M sucrose, 0.02M EDTA, and 0.1M imidazole) for determination of Na^+K^+ATPase activity. A further 50 mg of gill tissue was removed and placed in ice cold HET (0.02M Hepes, 0.001M EDTA, and 0.1% Triton X-100) for the determination of citrate synthase activity. All samples were stored at −80°C prior to analysis.

In 1991 and 1992 the same recirculating saltwater system was used to assess saltwater tolerance. The protocol for sampling, however, was modified to include measurement of plasma cortisol concentration. On May 19 and 26, 1991, and May 17, 1992 wild fish were caught during their nocturnal migration as they passed through the downstream enumeration fence on the Quinsam River. The following day, their hatchery counterparts were randomly sampled from the top and bottom sections of the earthen channel. Some fish were sacrificed immediately and others were transferred directly to salt water. For the initial measurement ($T = 0$), all fish were rapidly transferred after capture into a bucket of tricaine methanesulphonate (200 $mg \cdot L^{-1}$, MS-222) buffered with sodium bicarbonate (400 $mg \cdot L^{-1}$) to prevent the stress-related rise in cortisol following netting (Barton et al. 1986). After anaesthetizing, the caudal peduncle was severed, and the fish were bled and sampled as described above. Additionally, in 1991, the second gill arch was removed and placed in 10% buffered formalin for determination of chloride cell concentration on the primary lamellae. In 1991, fish were sampled after 0, 1, 2, 3, 7, and 14 d in salt water. In 1992, fish were sampled after 0, 1, and 7 d in salt water. Salinity for the SWC was 29‰ in 1991 and 35‰ in 1992. Temperature ranged from 13.0 to 15.3°C during the day in 1991, and from 12.0 to 14.6°C in 1992.

Analysis

Plasma sodium samples (1990) were measured with a Shimadzu ion chromatograph, model HIC-21, in a 5 mM nitric acid mobile phase. The 1991 and 1992 plasma sodium samples were measured with a Perkin-Elmer Atomic Absorption Spectrophotometer, model 2380. Na^+K^+ATPase activity was measured following the method of Zaugg (1982). Citrate synthase activity was measured following the method of Suarez et al. (1986). Enzyme activities were expressed per milligram protein of the gill homogenate. Protein concentration was determined by the biuret method as outlined in Alexander and Ingram (1980). Plasma cortisol concentration was determined by radioimmunoassay using ^{125}I-cortisol (Incstar corporation). In 1990, samples from six fish were assayed for each parameter measured, whereas in 1991 and 1992, ten fish for each group were used. Mortalities during the 1992 SWC reduced the number of samples that could be taken after 7 d in salt water for the hatchery fish.

Histology

For consistency, the second gill arch from the left side was removed and placed in 10% buffered formalin immediately after killing and sampling the fish. After fixing in formalin, samples were dehydrated through a graded series of alcohol to xylene and then imbedded in paraffin blocks. The blocks were cut in 6–10 μm sections, mounted on slides, and stained with Harris's haematoxylin and eosin.

Each microscope slide was examined for interlamellar chloride cell density (the number of chloride cells located on the primary lamellae). Seven fields for each gill arch were selected randomly and examined to determine the number of chloride cells, and to calculate a mean for each fish. A grand mean was then calculated for each group ($n = 6$).

Statistical Analysis

Parameters measured were compared for significant differences using analysis of variance, followed by a Tukey's test, to determine significant differences among means. Linear regression analysis was performed on the plasma sodium concentrations against fish weight for fish held in water for 24 h. Statistical significance was determined to be at a level of $\alpha = 0.05$. All values are expressed as means ± 1 SE.

Results

Plasma Sodium Concentration

In all years, plasma sodium concentrations for fish in fresh water did not differ significantly among groups (Fig. 1). After 24 h in salt water, all groups of fish showed an increase in plasma sodium concentration. This elevation was significantly greater in the hatchery-reared groups (hatchery 1 and 2) than in the wild or colonized fish. Plasma sodium concentrations in the wild and colonized fish following 24 h exposure to salt water did not differ significantly (Fig. 1a). Plasma sodium concentration declined by 7 d to levels not significantly different from the freshwater values in the wild and colonized fish, but were still significantly elevated in the two hatchery groups. Trends observed in 1990 were also seen in the 1991 and 1992 SWC tests (Figs. 1b–1d). Plasma Na^+ concentration showed a significantly greater perturbation in the hatchery fish than in the wild fish, and the perturbation

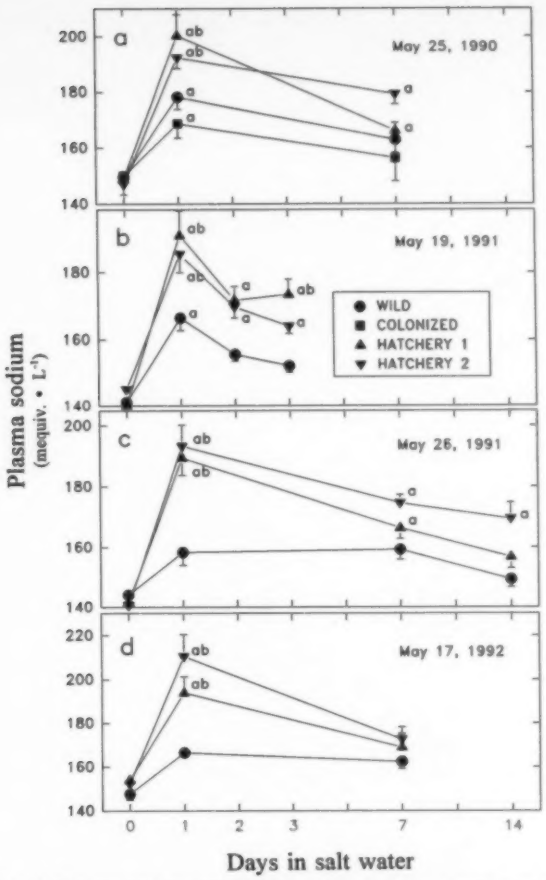

FIG. 1. Plasma sodium concentration for smolting juvenile coho salmon following direct transfer to salt water. Initial measurements (time = 0) were values for fish held in fresh water. The letter a adjacent to a symbol indicates that the value is significantly different from the time 0 value of the respective group, and the letter b adjacent to a symbol indicates that the value is significantly different from the wild fish value for the same time interval ($\alpha = 0.05$).

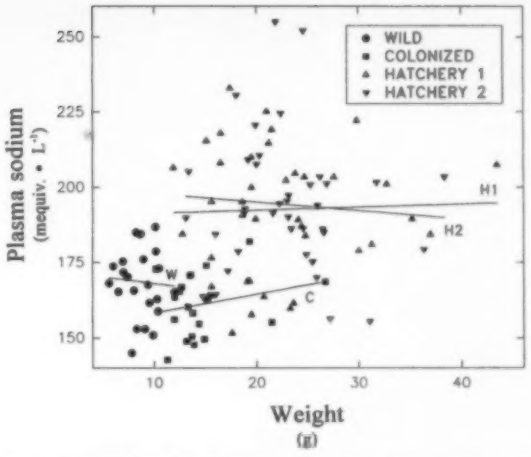

FIG. 2. The relationship between plasma sodium concentration (after 24 h in seawater) and fish weight. Linear regression lines are shown for each group. The R^2 values for the regression lines are 0.0035 (wild), 0.0508 (colonized), 0.0011 (hatchery 1), and 0.0049 (hatchery 2).

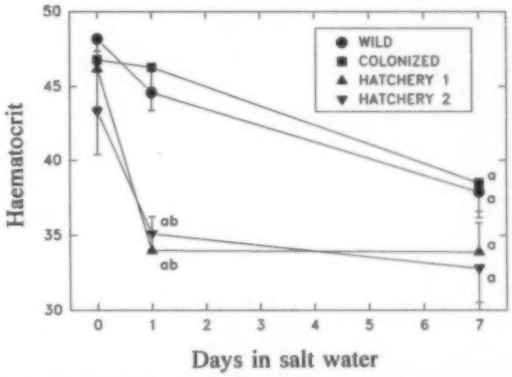

FIG. 3. Change in haematocrit following transfer to salt water for hatchery, wild, and colonized smolts in 1990. Letters indicate significant differences between groups, as described in Fig. 1.

persisted for a longer time. The May 26, 1991, plasma sodium concentrations for the wild fish after 24-h exposure to salt water did not show a significant change from freshwater levels (Fig. 1c). Despite the higher salinity of the SWC in 1992, wild fish still did not show a significant elevation in plasma sodium concentration following transfer to seawater (Fig. 1d).

There was no relationship between fish size within each group and the elevation in plasma sodium concentration following transfer to salt water for 24 h (Fig. 2). The slopes of the regression lines did not differ from zero for any of the groups.

Haematocrit

Declines in haematocrit following transfer to seawater were significantly greater in hatchery fish than in colonized and wild fish (Fig. 3). Haematocrits had declined significantly by 7 d in the colonized and wild fish as well, and there was no significant difference among any of the groups at that point.

Plasma Cortisol

In fresh water the circulating plasma cortisol concentration was greatest in the wild fish (Fig. 4). Following 24 h of exposure to salt water, there was a significant increase in cortisol levels in hatchery fish (Fig. 4). The elevation in plasma cortisol concentration appeared to be dependent on the severity of the osmotic challenge, as hatchery fish that were challenged to 35‰ salt water in 1992 (Fig. 4c) exhibited a much greater elevation than fish challenged to 29‰ (Fig. 4a, b) the previous year. After 48 h in salt water, cortisol titers had declined to freshwater levels. A rapid increase in plasma cortisol concentration was not seen in wild fish. Wild fish initially had a significantly greater cortisol titer than hatchery fish, but this level gradually declined with time during the challenge.

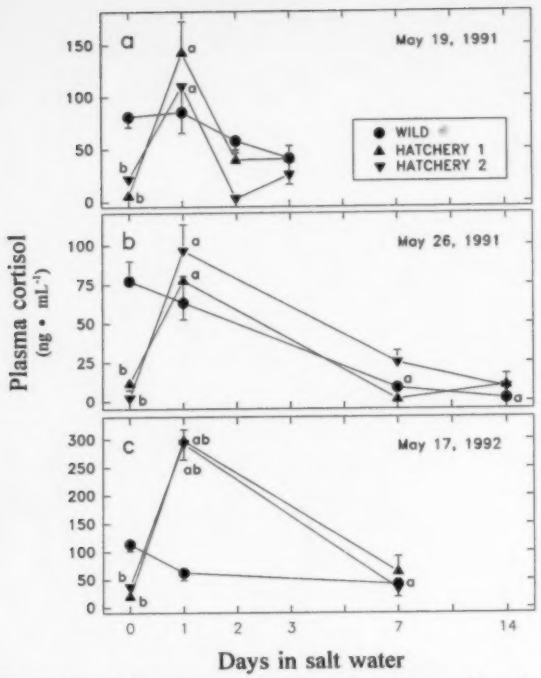

FIG. 4. Circulating plasma cortisol concentrations of hatchery and wild coho smolts before and after transfer to salt water. See Fig. 1 for a description of the symbols.

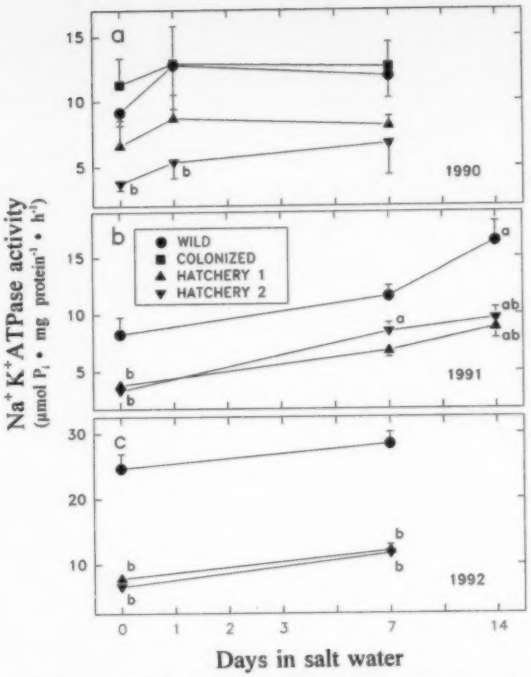

FIG. 5. Specific activity of Na^+K^+ATPase in the gills of hatchery, wild, and colonized coho salmon smolts before and after transfer to salt water for three consecutive years. See Fig. 1 for a description of the symbols.

Enzyme Activity

Specific activities of Na^+K^+ATPase were consistently lower in the hatchery groups (Fig. 5). In 1990, the difference was significant only between the colonized and hatchery 2 fish before and after 24 h in seawater (Fig. 5a). In 1991, the Na^+K^+ATPase activity showed a significant increase in all groups after 14 d in salt water (Fig. 5b). Owing to the increase in enzyme activity in the wild fish gills, there was still a significant difference in Na^+K^+ATPase activity between the wild and hatchery fish after 14 d. The difference in levels of citrate synthase activity in the gills was similar to that of Na^+K^+ATPase (Fig. 6). Gills from hatchery-reared fish had significantly lower enzyme activity than those from wild and colonized fish.

Chloride Cells

Chloride cell density on the primary lamellae was significantly greater in wild fish than in both hatchery groups prior to exposure to salt water (Fig. 7). Wild fish did not show an increase in chloride cell density during saltwater exposure. After 7 d in salt water, the number of chloride cells had significantly increased in both hatchery groups; however, the increase was significantly greater in hatchery 1 than hatchery 2 fish. By 14 d, there were no differences in chloride cell density among the three groups.

Figure 8 shows the relationship between Na^+K^+ATPase activity and chloride cell density in the gills for the 14-d SWC conducted in 1991. The two hatchery groups showed an increase in both chloride cell density and Na^+K^+ATPase activity, whereas the wild fish only showed an increase in Na^+K^+ATPase activity.

Mortality

In 1990, two hatchery 2 fish died after 24 h in salt water and one wild fish died after 5 d in salt water. There was no mortality due to saltwater exposure in the fish sampled during 1991. At the higher salinity (35‰) of the SWC conducted in 1992, approximately 25% and 30% of hatchery 1 and 2 fish died over a 1-wk period, respectively, but no wild fish died throughout the study period. The cumulative mortality for the wild and hatchery fish for the 1992 SWC is shown in Fig. 9.

Discussion

In this study, specific activity of Na^+K^+ATPase and chloride cell concentration indicated that wild fish were physiologically more advanced in the parr–smolt transformation when compared with hatchery-reared juvenile coho. Consequently, hatchery fish consistently showed a lower hypo-osmoregulatory ability compared with either wild or colonized fish. Both hatchery-reared groups exhibited much greater perturbations in plasma sodium concentration following seawater transfer than wild smolts (Fig. 1). Large elevations in plasma sodium reflect the fact that fish do not possess adequate physiological ability to hypo-osmoregulate in seawater (Blackburn and Clarke 1987).

The rapid change in haematocrit following transfer to salt water also indicated that hatchery fish experienced a large osmotic perturbation (Fig. 3). Blackburn and Clarke (1987) also reported a decrease in haematocrit after 24-h exposure to salt water, and attributed the reduction to dehydration of

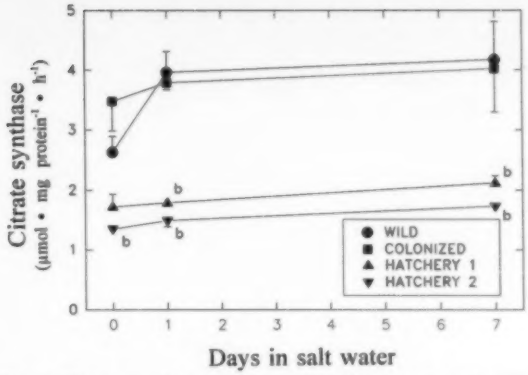

FIG. 6. Citrate synthase activity in the gills of coho salmon during a saltwater challenge conducted in May 1990. Symbols as in Fig. 1.

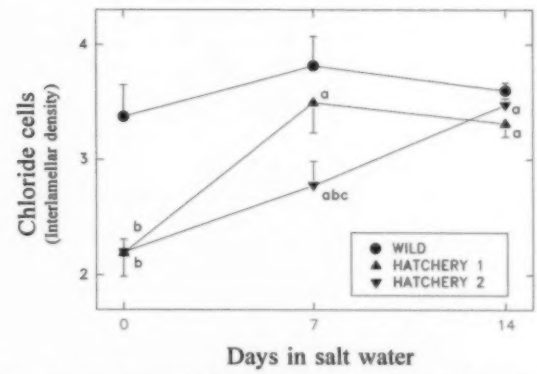

FIG. 7. The change in interlamellar chloride cell density for hatchery and wild coho smolts following transfer to salt water for 14 days in 1991. The letter c adjacent to a symbol indicates that the value is significantly different from the hatchery 1 value for the same time interval ($\alpha = 0.05$). All other symbols are as described in Fig. 1.

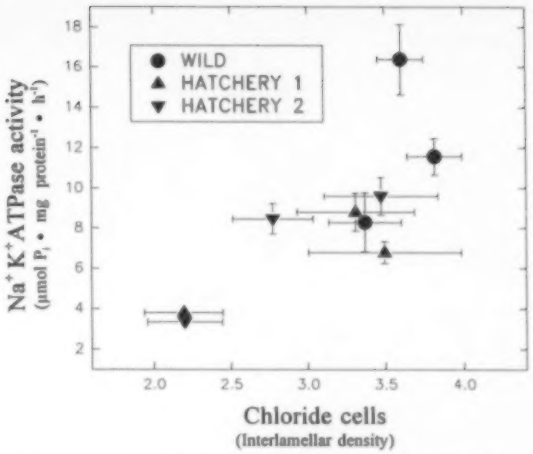

FIG. 8. The relationship between Na^+K^+ATPase activity and chloride cell concentration in the gills of hatchery and wild coho salmon when exposed to salt water for 14 d. Data are from a salt water challenge conducted in 1991.

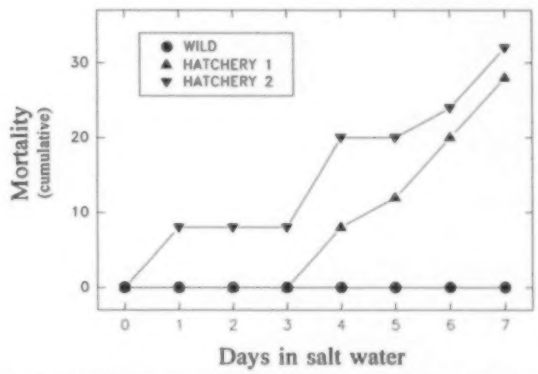

FIG. 9. Cumulative mortality for hatchery and wild coho salmon challenged with 35‰ salt water for 7 d in 1992.

the red blood cells. Similar reductions in haematocrit were also reported by Brauner et al. (1992) who speculated that an increase in plasma volume could account for the decline in haematocrit.

Greater saltwater tolerance of wild smolts is associated with higher activities of the enzymes Na^+K^+ATPase (Fig. 5) and citrate synthase (Fig. 6) in the gills. Na^+K^+ATPase is the primary enzyme for excretion of NaCl in saltwater teleosts (Epstein et al. 1980). Citrate synthase activity also increases during the spring, possibly to meet the energetic demand associated with cellular differentiation that occurs during smolt development (McCormick et al. 1989). Wild fish had higher Na^+K^+ATPase and citrate synthase activities, and greater concentrations of chloride cells on the primary lamellae than both groups of hatchery-reared fish. Greater activities of these enzymes and the concentration of chloride cells responsible for excretion of Na^+ and Cl^- across the gills in a saltwater teleost would reduce the osmotic perturbation in the plasma of fish during migration into the ocean.

Rapid transfer to salt water is an extreme test to measure a fish's ability to tolerate a hyperosmotic challenge. Normally, fish would migrate downstream and then into the estuary to reside, possibly allowing the fish additional time to adapt fully to the increase in salinity. Both distance and time of migration are important to smolt development, as coho and chinook juveniles show physiological changes with distance migrated downstream (Zaugg et al. 1985). It is possible, therefore, that given a greater opportunity to migrate, hatchery-reared coho salmon would probably compare more favourably with wild fish in ability to adapt to salt water. The Quinsam River Salmonid Hatchery, however, is located less than 5 km from the estuary. The hatchery-reared coho salmon released only a short distance from the estuary may require residence in the estuary for several days or weeks to develop adequately. Levings et al. (1986) found that wild juvenile chinook smolts reside in the estuary for 40–60 d, whereas hatchery smolts used the estuary for approximately half that time. The importance of residence in an estuary to the survival of smolts has been shown by Macdonald et al. (1988). Large hatchery coho, however, were found not to utilize the estuary extensively, but to inhabit deeper, more saline waters further from shore than their smaller wild counterparts (Argue et al. 1986; Macdonald et al. 1987). Macdonald et al.

(1987) speculated that the larger animals were more likely to be physiologically prepared for movement to the marine environment than smaller salmon. Our study indicates that the larger hatchery juvenile coho showed a weaker hypo-osmoregulatory ability than smaller wild smolts. Consequently migration of hatchery smolts directly into more saline, outer estuary would not be beneficial for survival. The location of the hatchery smolts in the outer estuary may be due to a preference for higher salinity water which may develop even though the smolts are not well adapted for the marine environment (Wedemeyer et al. 1980).

After transfer to salt water, there is an initial crisis period where plasma Na^+ levels become elevated. On the basis of ion levels, it would appear that the animals are fully acclimated to salt water by approximately the 7th day. There is evidence, however, that complete physiological adaptation to salt water may take substantially longer. Zaugg and McLain (1970) found that coho salmon smolts transferred into salt water take approximately 30–35 d for Na^+K^+ATPase activity to reach a maximum. We have presented evidence that the Na^+K^+ATPase activity may not have reached a maximum by 2 wk (Fig. 5). In contrast, chloride cell concentrations do appear to be maximal within 2 wk after transfer to salt water (Fig. 7). This is clear for wild coho, where there is no increase in chloride cell concentration following transfer to seawater, but Na^+K^+ATPase increased significantly (Fig. 8). Hatchery smolts showed an increase in chloride cell density and Na^+K^+ATPase activity following transfer to salt water. Therefore, complete adaptation to salt water may take more than 2 wk even though the fish appear to be adapted to salt water, as indicated by the stabilization of plasma sodium concentrations. The greater proliferation of chloride cells in hatchery 1 than hatchery 2 fish following 1 wk in salt water (Fig. 7), could be related to corticosteroid receptor concentration in the gills. As the fish in the hatchery 1 group possessed a greater number of corticosteroid receptors in the gills than hatchery 2, they would be expected to show a greater sensitivity to cortisol (Shrimpton et al. 1994). The surge in cortisol following seawater transfer may provide the stimulus necessary for an increase in chloride cell proliferation.

Based on saltwater tolerance, it is possible that entry into the marine environment is a time of high mortality. If there is not adequate time or space for fish to remain in the estuary, smolts that enter the marine environment directly will likely experience an elevation in plasma sodium concentration. Such a perturbation implies a reduction in physiological performance that could be manifested as a decrease in swimming performance. Brauner et al. (1992) showed that as plasma sodium concentration increased, the swimming ability of coho salmon decreased. Consequently, following seawater exposure the hatchery fish described in this study suffered a much greater decrease in swimming performance (Brauner et al. 1994) and could be more susceptible to predation.

There may be several reasons for differences in saltwater tolerance between fish reared in a wild environment and those reared in a hatchery environment. There is evidence that hatchery stocks have a significantly lower amount of genetic variability than wild populations (Waples 1991). It is possible that reduced genetic variability in a hatchery population may lead to reduced fitness (Danzmann et al. 1989), and may compromise the smolt's saltwater tolerance. However, colonized and hatchery fish used in this study were progeny of the same broodstock. As the colonized fish show as great a saltwater tolerance as the wild fish, it is not likely that reduced variability within the gene pool can account for the differences seen in the saltwater tolerance of wild and hatchery-reared coho salmon.

Selective pressures are continual for wild fish and survival from egg to smolt is less than 5% in Pacific salmonids (Waples 1991). In contrast, survival within a hatchery is very high (approximately 75%; Waples 1991). Colonized fish also encounter large mortality after transfer to the upper Quinsam watershed (W.E. McLean, Quinsam River Salmonid Hatchery, personal communication), thereby possibly encountering a selective pressure for the fittest individuals by thinning out the weakest hatchery fish. It is likely that the greatest mortality in colonized fish occurs soon after they are transplanted, but it is not clear whether such selective pressures at this early stage will select for traits that lead to good quality smolts and development of a strong saltwater tolerance in the spring.

An alternate hypothesis to account for the difference in smolting between hatchery and wild fish is that the rearing environment affects smoltification. In response to environmental stimuli, seasonal changes in structure and function occur prior to seawater exposure and are responsible for increased salinity tolerance during smoltification (McCormick and Saunders 1987). Photoperiod plays a large role in the timing and development of smolting in coho salmon (Clarke et al. 1981). The seasonal change in photoperiod was similar for both hatchery and wild fish in this study. There are, however, several stimuli experienced by wild fish during the spring that are either not present or are suppressed in a hatchery environment. Groundwater used to supply the ponds at the Quinsam River hatchery shows almost no change in temperature over the year, in contrast to the river. The change in ambient water temperature during the spring may be a stimulus for smoltification (Hoar 1988). The lack of an increase in water temperature may adversely affect smolting in hatchery fish. There does not appear, however, to be a relationship between smolt survival in the marine environment and whether juvenile salmonids are reared using either groundwater or surface water in the Salmonid Enhancement Program hatcheries of British Columbia (Cross et al. 1991).

Diet is vastly different between hatchery and wild fish. Hatchery fish are fed a high protein diet composed primarily of herring meal. Nutritional studies on hatchery fish have demonstrated that diet will affect growth, development, and marine survival of salmonids. Addition of polyunsaturated fatty acids to the diet improved the rate of smolt production in masu salmon, *O. masou* (Ogata and Murai 1989). Higgs et al. (1992) found that fatty acid composition affected smolt to adult survival in chinook salmon. Fatty acid composition in the feed altered the lipid composition of fish tissues. The change in membrane composition may play a role in gill function and ion transport (Van Praag et al. 1987). A comparison of fish reared in a seminatural environment with hatchery fish, however, did not indicate any difference in marine survival (Mundie et al. 1990). The diet of fish reared in a seminatural environment consisted of natural prey items that accounted for a significant proportion of the diet. Survival (as estimated by adult returns) of the seminatural smolts was similar or slightly lower than survival of the hatchery fish. Therefore, a wild diet was not shown to confer a significant survival advantage for coho smolts in that study.

Rearing conditions within the hatchery also affect smoltification. Increased rearing density causes a reduction in Na^+K^+ATPase activity in the gills (Strange et al. 1978; Patino et al. 1986), and reduced saltwater tolerance (Schreck et al. 1985). Increased rearing density during freshwater residence of hatchery coho has been shown to be inversely related to adult returns (Fagerlund et al. 1983). Rearing density within a hatchery is much greater than that experienced by wild fish in the river. Increasing rearing density to maximize production is stressful to fish, which may be due to social interaction (Peters et al. 1980; Ejike and Schreck 1980) or water quality degradation. An increase in suspended solids as nonfilterable residue (NFR) in the water will stress fish (Redding et al. 1987). Material comprising the NFR in the river is composed almost entirely of inorganic material whereas in hatchery water the NFR is composed of predominantly organic material that results from the accumulation of food and faecal matter and promotes bacterial production (K. Campbell, Quinsam River Salmonid Hatchery, personal communication). The high organic content of the NFR in the hatchery may be a source of physical and chemical damage to the gills (Banks et al. 1977). In this study, fish at the entrance to the rearing channel were exposed to much cleaner water than fish in the middle or bottom sections. This resulted in few physiological and anatomical differences between fish from the top and bottom sections of the earthen channel. As inflow water to the channel had previously supplied other parts of the hatchery, lack of a significant difference between hatchery 1 and 2 fish may indicate that the clarifiers were not effective in removing particulate matter.

As cortisol is both a stress hormone and a hormone responsible for initiating many of the physiological changes that are involved with smoltification, stress will have an effect on the smolting physiology of salmonids. Cortisol has a direct effect on the concentration of corticosteroid receptors in the gills of coho salmon. Both stress and exogenous treatment with cortisol were shown to cause a downregulation of the corticosteroid receptors in the gills (Shrimpton and Randall 1994). Shrimpton et al. (1994) found that the concentration of corticosteroid receptors in the gills was significantly lower in hatchery compared with wild fish, and proposed that hatchery fish were less sensitive to the surge in cortisol during the spring. Therefore, it is possible that stressful rearing practices within the hatchery lead to a downregulation of corticosteroid receptors in the gills and decrease the sensitivity of hatchery fish to the cortisol surge in the spring during smolt development. The lack of response to an environmental trigger for smolting can account for the reduced saltwater tolerance observed in the hatchery fish which, in turn, could account for the differences in survival between the wild and hatchery fish in this experiment.

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References

ALEXANDER, J.B., AND G.A. INGRAM. 1980. A comparison of five of the methods commonly used to measure protein concentrations in fish sera. J. Fish Biol. 16: 115–122.

ARGUE, A.W., B. HILLABY, AND C.D. SHEPARD. 1986. Distribution, timing, change in size, and stomach contents of juvenile chinook and coho salmon caught in Cowichan estuary and bay, 1973, 1975, 1976. Can. Tech. Rep. Fish. Aquat. Sci. 1431: 98 p.

BANKS, J.L., W.G. TAYLOR, AND S.L. LEEK. 1977. Carrying capacity recommendations for Olympia area national fish hatcheries. Completion Report for Abernathy Salmon Culture Technology Center, Washington. U.S. Fish and Wildlife Service, Washington, D.C.

BARTON, B.A., C.B. SCHRECK, AND L.A. SIGISMONDI. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Trans. Am. Fish. Soc. 115: 245–251.

BLACKBURN, J., AND W.C. CLARKE. 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. No. 1515: 35 p.

BLACKMUN, G.J., B.V. LUKYN, W.E. MCLEAN, AND D. EWART. 1985. Quinsam Watershed Study: 1983. Can. MS Rep. Fish. Aquat. Sci. 1832: 65 p.

BRAUNER, C.J., G.K. IWAMA, AND D.J. RANDALL. 1994. The effect of short-duration seawater exposure on the swimming performance of hatchery-reared and wild coho salmon (*Oncorhynchus kisutch*) juveniles during smoltification. Can. J. Fish. Aquat. Sci. 51: 2188–2194.

BRAUNER, C.J., J.M. SHRIMPTON, AND D.J. RANDALL. 1992. Effect of short-duration seawater exposure on plasma ion concentrations and swimming performance of coho salmon (*Oncorhynchus kisutch*) parr. Can. J. Fish. Aquat. Sci. 49: 2399–2405.

CLARKE, W.C., J.E. SHELBOURN, AND J.R. BRETT. 1981. Effect of artificial photoperiod cycles, temperature, and salinity on growth and smolting in underyearling coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*), and sockeye (*O. nerka*) salmon. Aquaculture 22: 105–116.

CROSS, C.L., L. LAPI, AND E.A. PERRY. 1991. Production of chinook and coho salmon from British Columbia hatcheries, 1971 through 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1816: 48 p.

DANZMANN, R.G., M.M. FERGUSON, AND F.W. ALLENDORF. 1989. Genetic variability and components of fitness in hatchery strains of rainbow trout. J. Fish Biol. 35(Suppl. A): 313–319.

EJIKE, C., AND C.B. SCHRECK. 1980. Stress and social hierarchy rank in coho salmon. Trans. Am. Fish. Soc. 109: 423–426.

EPSTEIN, F.H., P. SILVA, AND G. KORMANIK. 1980. Role of Na-K-ATPase in chloride cell function. Am. J. Physiol. 238: R246–R250.

FAGERLUND, U.H.M., J.R. MCBRIDE, B.S. DOSANJH, E.T. STONE, AND F.K. SANDERCOCK. 1983. Influence of culture density on juvenile coho salmon production and ocean survival. Can. Tech. Rep. Fish. Aquat. Sci. 1229: 27 p.

FISHER, J.P., AND W.G. PEARCY. 1988. Growth of juvenile coho salmon (*Oncorhynchus kisutch*) off Oregon and Washington, USA, in years of differing coastal upwelling. Can. J. Fish. Aquat. Sci. 45: 1036–1044.

HEALEY, M.C. 1982. Juvenile Pacific salmon in estuaries: the life support system, p. 315–341. *In* V.S. Kennedy [ed.] Estuarine comparisons. Academic Press, Toronto, Ont.

HIGGS, D.A., B.S. DOSANJH, M.D. PLOTNIKOFF, J.R. MARKERT, D. LAWSETH, J.R. MCBRIDE, AND J.T. BUCKLEY. 1992. Influence of dietary protein to lipid ratio and lipid composition on the performance and marine survival of hatchery reared chinook salmon (*Oncorhynchus tshawytscha*). Bull. Aquacult. Assoc. Can. 92: 46–48.

HOAR, W.S. 1988. The physiology of smolting salmonids, p. 275–344. *In* W.S. Hoar and D.J. Randall [ed.] Fish Physiology Vol. 11B. Academic Press, New York, N.Y.

LEVINGS, C.D., C.D. MCALLISTER, AND B.D. CHANG. 1986. Differential use of the Campbell River estuary, British Columbia, by wild and hatchery-reared juvenile chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 43: 1386–1397.

MACDONALD, J.S., I.K. BIRTWELL, AND G.M. KRUZYNSKI. 1987. Food and habitat utilization by juvenile salmonids in the Campbell River estuary. Can. J. Fish. Aquat. Sci. 44: 1233–1246.

MACDONALD, J.S., C.D. LEVINGS, C.D. MCALLISTER, U.H.M. FAGERLUND, AND J.R. MCBRIDE. 1988. A field experiment to test the importance of

estuaries for chinook salmon (*Oncorhynchus tshawytscha*) survival: short-term results. Can. J. Fish. Aquat. Sci. 45: 1366–1377.

McCormick, S.D., and R.L. Saunders. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. Am. Fish. Soc. Symp. 1: 211–229.

McCormick, S.D., R.L. Saunders, and A.D. MacIntyre. 1989. Mitochondrial enzyme and Na^+,K^+-ATPase activity, and ion regulation during parr–smolt transformation of Atlantic salmon (*Salmo salar*). Fish. Phys. Biochem. 6: 231–241.

Mundie, J.H., D.E. Mounce, and K.S. Simpson. 1990. Semi-natural rearing of coho salmon, *Oncorhynchus kisutch* (Walbaum), smolts, with an assessment of survival to the catch and escapement. Aquat. Fish. Manage. 21: 327–345.

Ogata, H., and T. Murai. 1989. Effects of dietary fatty acid composition on growth and smolting of underyearling masu salmon, *Oncorhynchus masou*. Aquaculture 82: 181–189.

Patino, R., C.B. Schreck, J.L. Banks, and W.S. Zaugg. 1986. Effects of rearing conditions on the developmental physiology of smolting coho salmon. Trans. Am. Fish. Soc. 115: 828–837.

Peters, G., H. Delventhal, and H. Klinger. 1980. Physiological and morphological effects of social stress in the eel, (*Anguilla anguilla* L.). Arch. Fischereiwiss. 30: 157–180.

Redding, J.M., C.B. Schreck, and F.H. Everest. 1987. Physiological effects on coho salmon and steelhead of exposure to suspended solids. Trans. Am. Fish. Soc. 116: 737–744.

Schreck, C.B., R. Patino, C.K. Pring, J.R. Winton, and J.E. Holway. 1985. Effects of rearing density on indices of smoltification and performance of coho salmon, *Oncorhynchus kisutch*. Aquaculture 45: 345–358.

Shrimpton, J.M., N.J. Bernier, and D.J. Randall. 1994. Changes in cortisol dynamics in wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. Can. J. Fish. Aquat. Sci. 51: 2179–2187.

Shrimpton, J.M., and D.J. Randall. 1994. Downregulation of corticosteroid receptors in the gills of coho salmon due to stress and cortisol treatment. Am. J. Physiol. 267: R432–R438.

Strange, R.J., C.B. Schreck, and R.D. Ewing. 1978. Cortisol concentration in confined juvenile chinook salmon (*Oncorhynchus tshawytscha*). Trans. Am. Fish. Soc. 107: 812–819.

Suarez, R.K., G.S. Brown, and P.W. Hochachka. 1986. Metabolic sources of energy for hummingbird flight. Am. J. Physiol. 251: R537–R542.

Van Praag, D., S.J. Farber, E. Minkin, and N. Primor. 1987. Production of eicosanoids by the killifish gills and opercular epithelia and their effect on active transport of ions. Gen. Comp. Endocrinol. 67: 50–57.

Walters, C. 1988. Mixed-stock fisheries and the sustainability of enhancement production for chinook and coho salmon, p. 109–115. *In* W.J. McNeil [ed.] Salmon production, management, and allocation. Biological, economic, and policy issues. Oregon State University Press, Corvalis, Oreg.

Waples, R.S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. Can. J. Fish. Aquat. Sci. 48(Suppl. 1): 124–133.

Wedemeyer, G.A., R.L. Saunders, and W.C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42: 1–14.

Zaugg, W.S. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aquat. Sci. 39: 215–217.

Zaugg, W.S., and C.V.W. Mahnken. 1991. The importance of smolt development to successful marine ranching of Pacific salmon. NOAA Tech. Rep. NMFS 102: 89–97.

Zaugg, W.S., and L.R. McLain. 1970. Adenosinetriphosphatase activity in gills of salmonids: seasonal variations and salt water influence in coho salmon *Oncorhynchus kisutch*. Comp. Biochem. Physiol. 35: 587–596.

Zaugg, W.S., E.F. Prentice, and F.W. Waknitz. 1985. Importance of river migration to the development of seawater tolerance in Columbia River anadromous salmonids. Aquaculture 51: 33–47.

Changes in Cortisol Dynamics in Wild and Hatchery-Reared Juvenile Coho Salmon (*Oncorhynchus kisutch*) During Smoltification

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Cortisol dynamics were examined in hatchery and wild juvenile coho salmon (*Oncorhynchus kisutch*) over the spring of 1991 and 1992 when the fish were smolting. Wild fish were caught in the upper Quinsam River, British Columbia and at the downstream enumeration fence when they started to migrate. Two groups of hatchery fish were from the top and bottom ends of an earthen rearing channel. Plasma cortisol levels increased during the spring in all groups, but were significantly greater in wild than in hatchery smolts. The half-life of plasma cortisol decreased as fish smolted; however, there was no difference among the groups. Corticosteroid receptor (CR) concentration and affinity decreased during the spring. Wild fish consistently possessed the greatest gill concentration of CR. The change in dissociation constant (k_D) was similar for hatchery and wild fish during spring 1991. In 1992, hatchery fish showed a similar gradual increase. In contrast, wild fish did not show an increase in k_D until May. The changes in cortisol concentration in the plasma and the CR k_D occurred synchronously with the increase in Na^+K^+ATPase activity in wild fish, which showed the greatest increase in k_D, plasma cortisol, and Na^+K^+ATPase activity.

Au cours des printemps 1991 et 1992, au moment de la smoltification, la dynamique du cortisol a été examinée chez des juvéniles d'élevage et à l'état sauvage du saumon coho (*Oncorhynchus kisutch*). Les sujets à l'état sauvage ont été capturés dans la partie amont de la rivière Quinsam, Colombie-Britannique, ainsi qu'à la barrière de dénombrement en aval lors du commencement de la migration. Deux groupes de poissons d'élevage, provenant des deux extrémités d'un bassin d'alevinage en terre, ont été étudiés. La concentration en cortisol plasmatique s'est accrue au printemps chez tous les groupes, mais elle était significativement supérieure chez les saumoneaux à l'état sauvage par rapport aux sujets d'élevage. La demi-vie du cortisol plasmatique s'est abaissée lorsque les poissons sont passés par le stade de la smoltification; il n'y avait cependant pas de différence entre les groupes. La concentration en récepteurs de corticostéroïdes (CR) et leur affinité ont diminué pendant le printemps. Les poissons à l'état sauvage ont toujours eu la concentration la plus élevée de CR dans leurs branchies. Le changement observé dans le cas de la constante de dissociation (k_D) a été le même chez les sujets d'élevage et chez les sujets à l'état sauvage durant le printemps 1991. En 1992, cependant, les poissons d'élevage ont marqué une hausse graduelle similaire. Par contre, les poissons à l'état sauvage n'ont montré aucune augmentation de la k_D avant mai. Les changements dans la concentration du cortisol plasmique et de la k_D des CR, se sont produits en même temps que l'augmentation d'activité de la Na^+K^+ATPase chez les poissons à l'état sauvage, chez qui on a observé la plus forte augmentation de la k_D, du cortisol plasmique et de l'activité de la Na^+K^+ATPase.

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The development of saltwater tolerance in Pacific salmonid smolts is affected by the rearing environment. Juvenile coho salmon (*Oncorhynchus kisutch*) reared in high density and reduced water flow showed impaired smolting as characterized by reduced saltwater tolerance (Schreck et al. 1985; Patino et al. 1986). Strange et al. (1978) showed that increases in rearing density resulted in significantly reduced Na^+K^+ATPase activities in juvenile chinook salmon (*O. tshawytscha*). Fish that exhibit large increases in Na^+K^+ATPase activity over the spring are classified as good-quality smolts, and show high smolt-to-adult survival rates (coho, Wahle and Zaugg 1982; chinook, Zaugg 1989). Consequently, the numbers of returning adult salmon can be partially dependent on the rearing environment of juveniles. Increased rearing density during the freshwater residence of hatchery coho has also been shown to be inversely related to adult returns (Fagerlund et al. 1983). Other factors associated with rearing environment in the hatchery also affect adult survival. Numbers of returning adult chinook salmon differed between sections of an earthen channel. Fish from the top section showed a greater smolt-to-adult survival than those from the bottom section of the same channel (W.E. McLean, Quinzam River Salmonid Hatchery, Box 467, Campbell River, BC V9W 5C1, personal

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communication). Therefore, the rearing environment during the early life history of salmonids can affect smolt development. Shrimpton et al. (1994) have shown that the saltwater tolerance of coho salmon is different between fish reared in the wild and those reared in the hatchery and that these differences could be attributed to differences in the rearing environment.

During the spring, juvenile salmon fry change from an animal adapted to a freshwater environment, to one capable of osmoregulating in the marine environment. The process of smoltification involves numerous physiological and structural modifications that are stimulated by hormonal changes (Hoar 1988). Cortisol is one of the major hormones involved in smolting, and has been shown to increase saltwater tolerance when administered to juvenile salmonids by causing an increase in chloride cell proliferation and Na^+K^+ATPase activity (coho, Richman and Zaugg 1987; rainbow trout (*O. mykiss*), Madsen 1990). Cortisol, however, is also released when fish are stressed. Stressors associated with the rearing environment will affect cortisol dynamics in juvenile salmonids. Stress has been shown to cause an increase in plasma cortisol concentration (Barton and Iwama 1991), as well as to decrease the half-life of cortisol in the plasma (Redding et al. 1984), and decrease the number of corticosteroid receptors in the gills (Maule and Schreck 1991; Shrimpton and Randall 1994). It is interesting that some of the documented changes in cortisol dynamics during smolting are similar to those that occur in response to a stressor (e.g., increases in plasma cortisol, Sprecker and Schreck 1982; Barton et al. 1985; decrease in half-life of cortisol in the plasma, Patino et al. 1985).

As there is a large difference in the saltwater tolerance of juvenile coho salmon that are reared in a hatchery compared with those reared naturally in the river (Shrimpton et al. 1994), and there is much evidence that the rearing environment will affect smolting and cortisol dynamics, we examined differences in cortisol dynamics in both hatchery and wild coho salmon during the spring when the fish were smolting.

Materials and Methods

Fish

Three groups of juvenile coho salmon from the Quinsam River were examined over the spring of 1991 and 1992. The groups of animals used and the rearing conditions are described in Shrimpton et al. (1994). Briefly, the groups consisted of wild coho and two hatchery groups. The hatchery groups were taken from the top and bottom sections of an earthen rearing channel, and are designated hatchery 1 and hatchery 2, respectively. In 1991, the wild fish were caught in baited minnow traps. The traps were left in the river for up to 6 h, at which time the traps were rapidly removed from the water and the fish transferred to a bucket containing 200 $mg \cdot L^{-1}$ tricaine methanesulphonate (MS-222), buffered with 400 $mg \cdot L^{-1}$ sodium bicarbonate. This dosage of MS-222 has been shown to be sufficient to prevent the release of cortisol in fish following capture (Barton et al. 1986). The residence time in the minnow traps, however, proved to be stressful for the fish; thus, wild fish were captured by electrofishing in 1992. A sufficient voltage was used while electrofishing to stun the animals immediately, and by holding the fish in the electrical field it was possible to kill the animals very quickly. In 1991 and 1992, when smolts began to migrate, wild fish were caught at the downstream enumeration fence on the Quinsam River as described by Shrimpton et al. (1994). After sacrificing the fish, weight (g) and length (cm) were measured, the caudal peduncle was severed, and blood samples were collected in heparinized capillary tubes. The tubes were kept on ice until they could be centrifuged, and the plasma separated and collected. Plasma samples were frozen at −80°C until determination of cortisol and sodium concentrations. Gills were removed and treated as described below.

Hatchery fish were captured by seining the earthen channel. The seine was drawn toward the shore and fish collected from the seine were immediately transferred to a bucket containing MS-222 (1991), or a bucket with only water and sacrificed by electro-anaesthesia (1992).

Tissue Preparation for Corticosteroid Receptors

Gills were excised from the fish and blotted on tissues to remove excess blood and then placed in a solution of 10 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol, 20 mM sodium molybdate, and 10% (vol/vol) glycerol all at pH 7.4 (TEMS). Approximately 0.5 g of gill tissue was added to 2.5 mL of TEMS and stored in liquid nitrogen until samples were assayed for corticosteroid receptors (CR). Tissues from up to eight individuals were pooled for one sample. Four samples were assayed for CR for the hatchery groups per sampling interval. Capture of a large number of wild fish was often very difficult. Consequently, enough tissue for four CR samples could not always be obtained and less than four wild samples were assayed on several occasions. The method of Maule and Schreck (1990) was used to assay for the receptors. All procedures were carried out at 4°C by keeping samples on ice. Tissue was chopped finely and then homogenized using an IKA-Ultra Turrax TP 18/10S1 homogenizer for three 10-s bursts. Homogenates were centrifuged in a Sorvall RC5C centrifuge with a SS-34 rotor at 2000 × *g* for 10 min to remove gross debris. The supernatant was removed and centrifuged at 100 000 × *g* for 1 h in a Beckman L8-80 Ultracentrifuge with a SW-51 rotor. After this centrifugation, the supernatant was removed mixed with an equal volume of TEMS containing 5% (w/v) activated charcoal and 0.5% (w/v) dextran and incubated for 10 min to remove endogenous steroids. To separate charcoal from liquid, samples were centrifuged at 3500 × *g* for 20 min. The final supernatant was used to quantify cortisol binding. Protein content of this fraction was assayed according to the method of Bradford (1976), and if necessary buffer was added to adjust the protein content to 4–6 $mg \cdot mL^{-1}$.

Steroid Binding Assay

Cortisol binding receptor studies were conducted with [1,2-^{3}H]triamcinolone acetonide (TA; 1,4-pregnadien-9α-fluoro-11β,16α,-17α,21-tetrol-3,20-dione-16,17 acetonide) with a specific activity of 57.9 $Ci \cdot mmol^{-1}$ (Dupont NEN). TA was used throughout the study as Maule and Schreck (1990) showed that, in binding and competition studies on duplicate gill homogenates, TA and cortisol appeared to bind to identical receptor populations, except that TA bound to the receptor with a higher affinity. To determine the number of high affinity cortisol receptors, 100 μL of the final supernatant was incubated in duplicate aliquots with 100 μL

of buffer containing [^{3}H]TA with and without a 200-fold excess of cold TA. Final concentration of [^{3}H]TA ranged from 0.1 to 9 nM. Tubes were vortexed and incubated for 2 h. After the incubation period, 0.5 mL of TEMS containing 2.5% (w/v) activated charcoal and 0.25% (w/v) dextran was added and vortexed. After 10 min, charcoal containing unbound ligand was separated from bound ligand by centrifuging at 3000 × *g* for 20 min in a Beckman TJ6R refrigerated centrifuge. A 0.5-mL volume of the liquid was added to 5 mL of ACS (Amersham) aqueous counting scintillant. Samples were counted on a LKB 1214 Rackbeta liquid scintillation counter. Specific binding was determined by subtracting nonspecific bound from total bound. The equilibrium dissociation constant (k_D) and the concentration of corticosteroid receptor sites (B_{max}) were calculated according to Scatchard (1949). B_{max} was divided by the homogenate protein concentration, and CR concentration was expressed as fmol·mg protein^{-1}. The Hill coefficient was calculated according to Bennett (1978).

In 1991, the use of minnow traps to catch wild fish resulted in a significant stress response. Owing to high circulating plasma cortisol concentrations, receptors in the gills of wild fish were bound to the endogenous cortisol resulting in a proportion of the gill CR population not detected by the radioreceptor assay used in this study. Shrimpton and Randall (1994) showed a relationship for plasma cortisol and gill CR concentration and affinity. Using this relationship, we calculated the total concentration of gill CR that the fish would have possessed for a plasma cortisol concentration of 5 ng·mL^{-1} for the first three sampling intervals for wild fish in 1991. We assumed that a cortisol concentration of 5 mg·mL^{-1} was the resting value for wild fish at that time of the year as this level is similar to values measured in 1992 for wild fish.

Na^+K^+ATPase Activity

Gill arches from one side of the animal (approximately 50 mg) were removed and blotted dry on paper tissues. Gill tissue was placed in 0.5 mL of ice cold SEI (0.3 M sucrose, 0.02 M EDTA, and 0.1 M imidazole). Samples were frozen and stored at −80°C until analysis for enzyme activity following the method of Zaugg (1982). Ten samples were assayed for each sampling time, except for wild fish where sample size was limited to the number of fish that could be caught. Enzyme activities were expressed per mg protein in the gill homogenate. Protein was assayed using the Biuret method described by Alexander and Ingram (1980).

Plasma Cortisol Half-Life

Half-life of cortisol in the plasma of juvenile wild and hatchery coho was measured using a method similar to that of Redding et al. (1984). Wild juvenile coho were caught either by minnow trap or at the downstream enumeration fence, and transported to an aluminum trough at the Quinsam River Hatchery. Trapping was conducted over a 24-h period to obtain sufficient numbers of wild fish. Hatchery groups were seined from their respective sections of the earthen channel and also transported to an aluminum trough. Fish were left in the trough up to 24 h before estimation of cortisol half-life. Redding et al. (1984) showed that acute stress for 12 h does not affect the clearance of cortisol from the plasma, but 5 d of stress does. The change in rearing environment for the fish, and the stress associated with capture, is not likely to have affected plasma cortisol half-life.

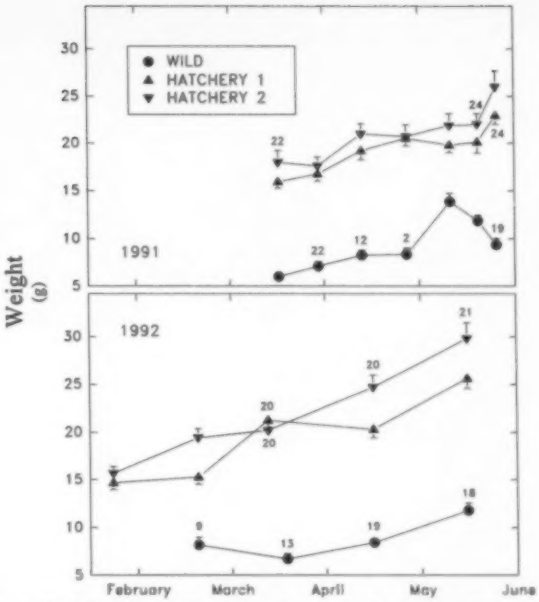

FIG. 1. Weight of juvenile coho salmon caught during 1991 and 1992 (n = 25 unless otherwise indicated above the data points). Wild fish were caught in the Quinsam River, and hatchery 1 and 2 fish were seined from the top and bottom sections of an earthen rearing channel, respectively. Hatchery 1 and 2 fish were significantly larger than wild fish at all sampling intervals.

To determine cortisol half-life in plasma, fish were removed from the aluminum trough and anaesthetized using buffered MS-222 (50 mg·mL^{-1}). Up to 32 fish from each group were injected with 0.05 μCi·g^{-1} (9 ng·mL^{-1}) of [1,2-^{3}H]cortisol (Dupont-NEN) in a 5% ethanol/saline solution intracardially. After 1.5, 3, 4.5, and 6 h, five to eight fish were killed with a lethal dose of MS-222, the caudal peduncle was severed, and duplicate blood samples were collected in heparinized capillary tubes. The tubes were centrifuged at 11 500 × *g* for 5 min and 20 μL of plasma was placed into a 20 mL scintillation vial. Five millimetres of aqueous scintillation cocktail (ACS; Amersham) were added to each vial and radioactive decay of the samples was counted for 5 min in an LKB Rackbeta scintillation counter. The disappearance of radioactivity from the plasma over time was used to estimate the cortisol half-life. A plot of plasma radioactivity as a function of time after injection indicated an exponential loss of radioactivity from the plasma. For a one-compartment model of hormone distribution, the time course of disappearance from the plasma is represented by an exponential decay. Logarithmic transformation of the data resulted in a linear relationship, and the half-life of cortisol was calculated from the slope of the linear regression according to Normand and Fortier (1970). Fish that had not been successfully injected intracardially had significantly lower decay values and were excluded from calculation of plasma cortisol half-life (less than 8% of the injected fish).

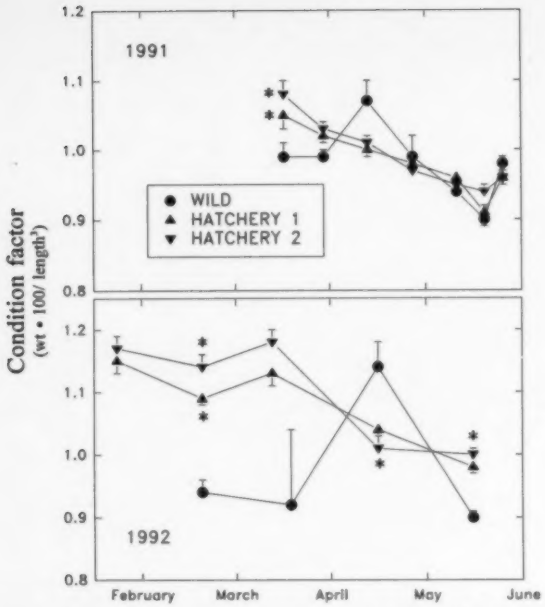

FIG. 2. Change in condition factor over the spring of 1991 and 1992 for wild and hatchery-reared coho salmon*. Significantly different ($\alpha = 0.05$) from wild fish values at the sampling interval. Groups are as described in Fig. 1. Weight and length were measured in g and cm, respectively.

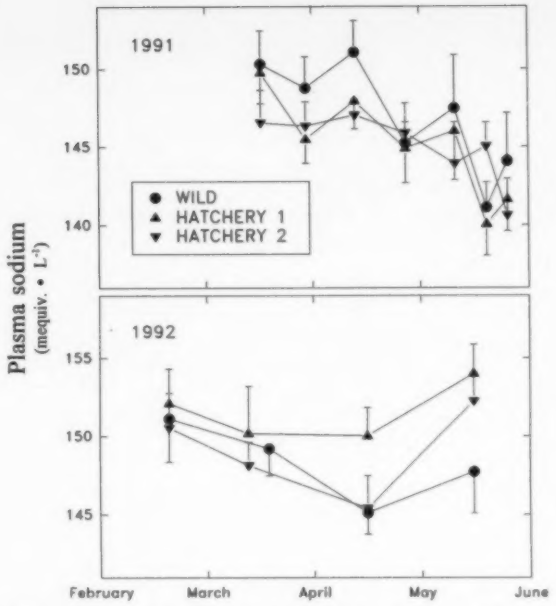

FIG. 3. Plasma sodium concentrations during the spring for hatchery and wild coho salmon ($n = 12$). Symbols and groups are as described in Fig. 1.

Analysis

Plasma sodium concentration was measured using a Perkin-Elmer Atomic Absorption Spectrophotometer, model 2380. Twelve samples were analyzed from each group. Plasma cortisol was measured using a radioimmunoassay kit using ^{125}I (Incstar Corp.). Ten samples were analyzed from each group.

Statistical Analysis

Condition factor (CF) was calculated as (weight/length^3) $\times$ 100. Fish weight, condition factor, plasma cortisol concentration, CR concentration and affinity, Na^+K^+ATPase activity, and plasma sodium concentration for the hatchery and wild fish were compared for significant differences using analysis of variance, followed by a Tukey's test to find significant differences among means. The half-life of cortisol in the plasma was compared for significant difference among groups and sampling intervals by analysis of covariance. Statistical significance was taken at a level of $\alpha = 0.05$, except where indicated otherwise. All values are expressed as means ± SE.

Results

Weight and Condition Factor

Fish sampled from the hatchery were significantly larger than the wild fish at all sampling intervals during the course of this study. Relative weight of fish are shown in Fig. 1.

Condition factor (CF) declined over the spring (Fig. 2). Although there was a significant reduction in CF over the spring, the pattern of decline was not the same for wild and hatchery fish. Over most of the year, wild coho exhibited lower CF than hatchery coho. This relationship was reversed in early April of 1991 and 1992, when CF was greater for wild than both groups of hatchery fish. There was a significant reduction in CF in the wild fish between April and May for both years of this study.

Plasma Sodium Concentration

Plasma sodium concentrations showed a gradual decline over the spring in 1991, but not in 1992 (Fig. 3). Analysis of variance performed on the 1991 data indicated that the decline was significant over time, but a Tukey's test showed that there was no significant difference among sampling dates.

Plasma Cortisol Concentration

In 1991, fish sampled from the hatchery 2 group showed a significant increase in plasma cortisol concentration with one point at the beginning of May, whereas the fish sampled from the hatchery 1 group did not show a significant increase in plasma cortisol over earlier spring measurements (Fig. 4). In 1991, cortisol concentrations in wild fish were always much higher than hatchery fish. For the first three sampling periods, wild fish were caught in minnow traps, which resulted in a considerable stress-associated rise in cortisol; these values were omitted from Fig. 4. Plasma cortisol concentrations were 149 ± 29, 130 ± 25, and 62 ± 16 for the March 16, 29, and April 12, 1991 sampling dates, respectively. During the last three sampling periods, wild fish were captured at the downstream enumeration fence and were anesthetized in MS-222 immediately after capture to prevent any stress-associated rise in cortisol. During this time cortisol concentrations in wild fish were significantly greater than in hatchery fish. In 1992, a similar trend was observed. The increase in plasma cortisol was significantly greater in late April than in March for both groups of hatchery smolts. Wild fish had significantly lower cortisol levels

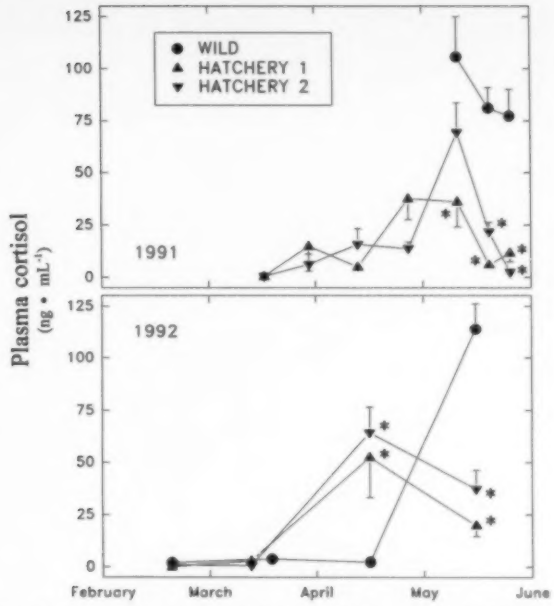

FIG. 4. Changes in concentrations of plasma cortisol of hatchery and wild juvenile coho salmon from the Quinsam River over the spring ($n = 10$). *Significantly different ($\alpha = 0.05$) from wild values at the same sampling interval. Symbols and groups are as described in Fig. 1.

in April than hatchery fish. The highest plasma cortisol levels occurred in May for the wild fish. The maximum cortisol concentration achieved during the spring was significantly greater in the wild smolts (May) than in either of the hatchery groups (April).

Plasma Cortisol Half-Life

During the spring, the half-life of cortisol in the plasma showed a decline in all groups of fish examined (Fig. 5). By May, the half-life of cortisol was significantly less than March values. For each sampling date, however, there was no significant difference in plasma cortisol half-life among groups.

Corticosteroid Receptors

Figure 6 shows representative saturation plots and Scatchard plots for the Quinsam River coho examined in this study. The data presented are for two sampling intervals during the year on March 12 and May 15 of 1992 for Hatchery 1 fish. B_{max} and k_D values were 105 ± 8.5 fmol·mg protein^{-1} and 0.56 ± 0.04 nM, respectively, for the March sampling period and 52 ± 8.4 fmol·mg protein^{-1} and 0.79 ± 0.01 nM, respectively, for the May sampling period. Hill coefficients were 1.01 ± 0.01 and 1.04 ± 0.02 for March and May sampling intervals, respectively. The concentration of corticosteroid receptors in the gills declined over the spring as fish smolted (Fig. 7). Wild fish consistently had the greatest concentration of corticosteroid receptors in the gills. Hatchery 1 fish had slightly higher concentrations than hatchery 2 fish. In 1991, there was a gradual increase in the dissociation constant (k_D) in both wild and hatchery-reared fish (Fig. 8). In 1992, hatchery fish showed a similar gradual increase in k_D. Wild fish did not show an increase from February to

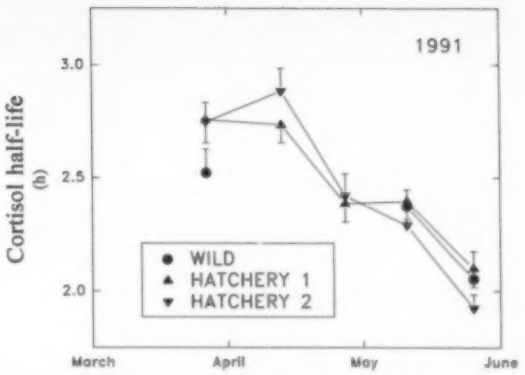

FIG. 5. Half-life or cortisol in the plasma of hatchery and wild juvenile coho salmon from the Quinsam River during the spring. Symbols and groups are as indicated in Fig. 1.

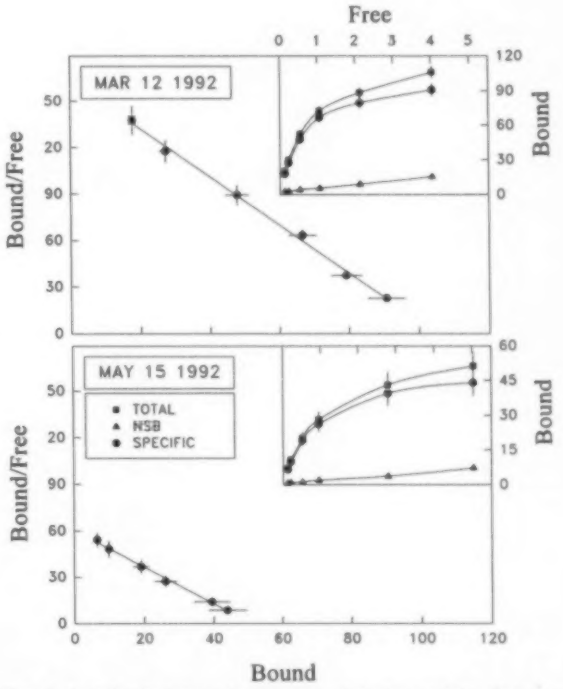

FIG. 6. Scatchard plots and saturation plots (inserts) for Quinsam River coho salmon gill cytosol bound to [^{3}H]triamcinolone acetonide. Data are for hatchery 1 coho on March 12 and May 15 1992. Units are fmol·mg protein^{-1} (bound) and nM (Free).

April but exhibited a large increase in k_D from April to May. The temporal change in k_D meant that the affinity of CR for cortisol in the wild fish was significantly different from the two hatchery groups. The Hill coefficients did not differ significantly from 1.0 for all the samples analyzed.

Na^+K^+ATPase Activity

Wild fish showed a significant increase in specific activity of Na^+K^+ATPase in the gills during May of both years (Fig. 9). Although, the enzyme activity was greater in gills of hatchery fish in May than the preceding months, levels did

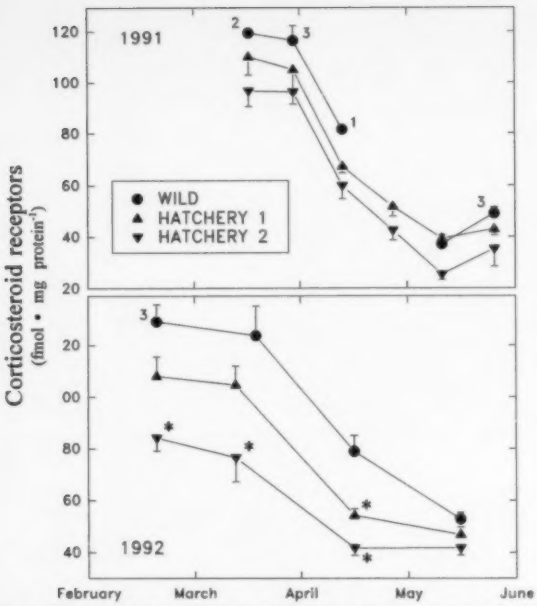

FIG. 7. Concentration of corticosteroid receptors in the gills of hatchery and wild coho salmon during the spring of 1991 and 1992 from the Quinsam River. The first three data points for the wild fish in 1991 were calculated to correct the high circulating cortisol levels of the plasma ($n = 4$ unless otherwise indicated adjacent to the data points). *Significantly different ($\alpha = 0.05$) from wild values at the sampling interval. Symbols and groups are as described in Fig. 1.

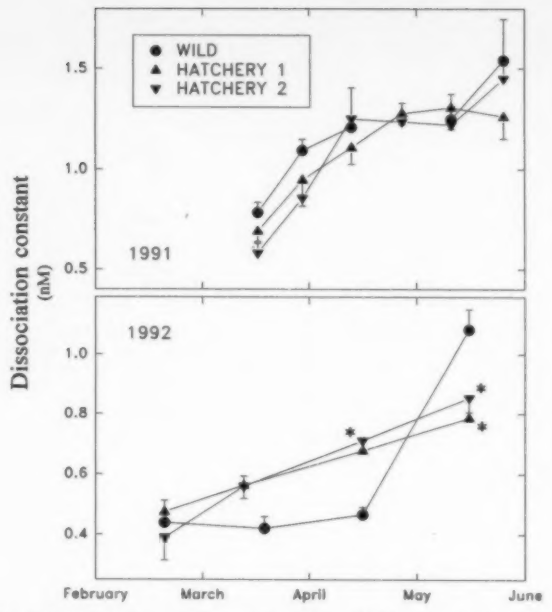

FIG. 8. Dissociation constant of corticosteroid receptors in gills of hatchery and wild coho salmon during the spring (numbers are as indicated in Fig. 7). *Significantly different ($\alpha = 0.05$) from wild values at the sampling interval. Symbols and groups are as described in Fig. 1.

not differ significantly. Consequently, during May Na^+K^+ATPase activity was significantly greater in wild fish compared with hatchery fish.

The activity of Na^+K^+ATPase was plotted against circulating plasma cortisol concentrations measured for all sampling dates of 1991 and 1992 except those from the wild fish caught in the minnow traps (Fig. 10). Wild fish exhibited the greatest increase in Na^+K^+ATPase activity for a given increase in plasma cortisol concentration. Hatchery 2 fish showed no increase in Na^+K^+ATPase activity with an increase in plasma cortisol concentration. The slopes of linear regressions were 0.101, 0.018, and 0.001 units of Na^+K^+ATPase activity per unit plasma cortisol concentration for wild, hatchery 1, and hatchery 2 groups, respectively. The regression lines did not differ significantly from zero for the two hatchery groups, but showed a significant relationship ($\alpha = 0.10$) for the wild fish.

Discussion

During the spring, both hatchery and wild juvenile coho showed many morphological and biochemical changes that indicated the fish were smolting. A decrease in CF has long been associated with parr–smolt transformation (McCormick and Saunders 1987). Hatchery fish showed a gradual reduction in CF throughout the spring (Fig. 2). In contrast, the pattern was more variable in wild fish, but showed a dramatic drop between April and May. The difference in smolt development between the groups of fish was also seen in the timing of colour change. Some of the hatchery-reared fish began to turn silver in March, and the majority of the fish sampled in April were silver and appeared to have smolted. The wild fish sampled in April all exhibited pronounced parr marks, and it was not until May that wild fish showed any signs of silvering. These morphological observations indicate that the temporal physiological changes associated with smolting were not the same for hatchery and wild coho salmon.

Another parameter that has been found to represent a physiological change associated with smolting is a decline in plasma sodium concentration (McCormick and Saunders 1987), which was seen in hatchery and wild smolts in 1991, but not in 1992. There was no difference, however, between wild and hatchery coho plasma sodium concentrations for any sampling interval.

Several changes in cortisol dynamics occurred as the fish smolted during the spring. There was an increase in plasma cortisol concentrations (Fig. 4), a decrease in the half-life of cortisol in the plasma (Fig. 5), and a decline in the number (Fig. 7) and affinity (Fig. 8) of the receptors for cortisol in the gills. Although the pattern of changes in cortisol dynamics were similar for hatchery and wild fish, the magnitude of the changes was often greater in wild fish than in hatchery fish. The suppressed changes in cortisol dynamics in the hatchery-reared fish were associated with no significant rise in gill Na^+K^+ATPase activity. As Na^+K^+ATPase activity in the gills has been correlated with the development of saltwater tolerance in smolts, hatchery-reared coho would not be expected to adapt to the increased salinity of the marine environment as well as the wild smolts. In a companion study, the hatchery smolts showed a weaker saltwater tolerance than their wild counterparts of the same stock

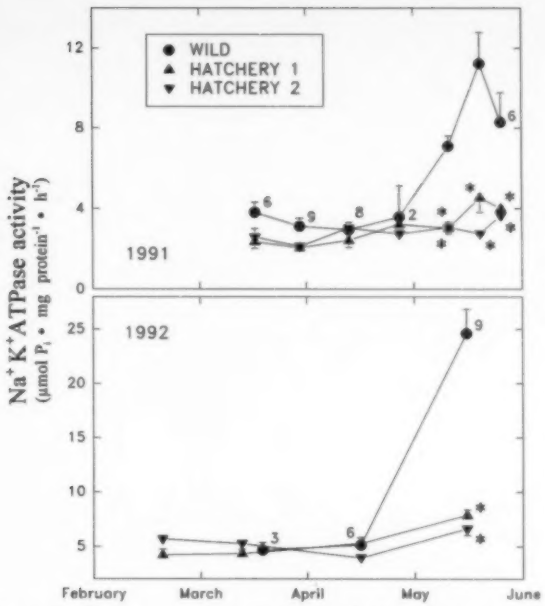

FIG. 9. Changes over the spring in Na^+K^+ATPase activity in gills of juvenile hatchery and wild coho from the Quinsam River (n = 10 unless indicated adjacent to the data points). *Significantly different (α = 0.05) from wild values at the same sampling interval. Symbols and groups are as described in Fig. 1.

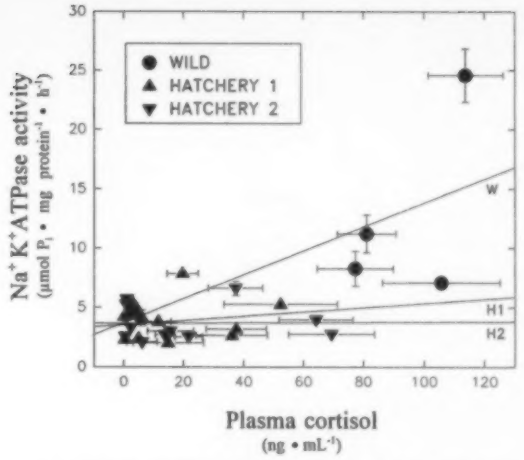

FIG. 10. The relationship between gill Na^+K^+ATPase activity and cortisol concentration in the plasma of juvenile hatchery and wild coho salmon. Linear regression lines for wild (W), hatchery 1 (H1), and hatchery 2 (H2) are shown on the plot.

(Shrimpton et al. 1994). Reduced hypo-osmoregulatory ability in hatchery fish may result from genetic differences among the groups or environmentally induced modifications in development. There is not likely to be a significant difference in hatchery and wild fish genetically. Field observations conducted by the hatchery personnel indicate that adults of hatchery origin migrate along with adults that originate from fry stocking and natural spawning into the Quinsam River above the enumeration fence (R. Kraft, Quinsam River Salmonid Hatchery, personal communication). Wild smolts produced in the Quinsam River could be the progeny of either hatchery or wild adults. Consequently, the differences seen between juvenile wild and hatchery coho in the present study is attributed to rearing environment. Rearing salmonids in intensive fish culture facilities has resulted in the fish being exposed to a number of stresses that are either not experienced in the natural environment at all or to the same degree (Donaldson 1981). Stress greatly affects plasma cortisol levels (Barton and Iwama 1991), half-life of cortisol in the plasma (Redding et al. 1984), and the concentration of corticosteroid receptors in the gills (Maule and Schreck 1991; Shrimpton and Randall 1994).

The morphological, biochemical, and physiological changes in the wild smolting juvenile coho appeared to occur synchronously. Between April and May a marked decline in CF and change to the silvery appearance of a smolt occurred at the same time that Na^+K^+ATPase activity showed a significant increase, plasma cortisol was at the highest level, and k_D of gill CR increased. These physiological changes were closely timed with the onset of migration to the marine environment. The wild smolts were able to hypo-osmoregulate well in salt water, and likely would have the best chance for survival. In contrast, many of the morphological and physiological events that occurred in the hatchery fish were not synchronous. There tended to be a more gradual change in physiology of the hatchery coho. The decrease in CF and the increase in k_D occurred more gradually over the spring, the change in plasma cortisol and colouration occurred much earlier, and there was an insignificant increase in gill Na^+K^+ATPase activity. These changes appeared to be accelerated in hatchery fish, but did not develop to the same degree as in wild fish. Consequently, the hatchery fish showed a reduced saltwater tolerance compared to their wild counterparts.

It is possible, however, that the synchronous changes that occur in the wild fish caught during this study could be partially attributed to sampling. For the May sampling period, the wild fish were caught in the downstream enumeration fence during migration. Only migrants were caught and the apparent synchronous timing of physiological and morphological changes in wild fish could be an artifact of sampling. Migration of wild smolts down the Quinsam River, however, is of short duration. Over 75% of the wild smolts migrate over a 12-d period (Blackmun et al. 1985). The fact that most of the wild fish migrate within a narrow time period and that there are few 2-year-old smolts in the river (<1%, M. Trenholme, Quinsam River Salmonid Hatchery, personal communication) is evidence that the protocol employed in the present study resulted in the capture of a representative sample of wild smolts. When given access to the river, hatchery smolts leave the rearing channel within 5 d; up to 90% of the fish leave within the first 48 h. It is estimated that fish reach the estuary within 48 h after entering the river (R. Kraft, Quinsam River Salmonid Hatchery, personal communication). Hatchery smolts were sampled just prior to release to obtain a representative sample for the population. The differences observed between timing of smolting in hatchery and wild coho in the present study is likely to be an accurate representation of differences in the two populations.

The increase in cortisol during the spring is a characteristic physiological change associated with smolting (Specker

and Schreck 1982; Hoar 1988). We found that the rearing environment has a pronounced effect on the spring rise in cortisol as the fish smolt, being significantly greater in wild than in hatchery fish. Sampling times, however, may have exaggerated this difference, as the wild fish were sampled during the night when increased cortisol levels have been observed (Thorpe et al. 1987; Pickering and Pottinger 1983; Rance et al. 1982), and the hatchery fish were sampled during the day. These researchers have seen a twofold increase at night, peaking at 24:00. Barton et al. (1986), however, did not observe any diurnal change in plasma cortisol concentration. This discrepancy could be attributed to feeding regimes, which have an important role on phasing of the corticosteroid rhythm (Bry 1982). Fish given a single meal near the start of the light phase showed a corticosteroid peak during the hours of darkness, whereas feeding at other times or feeding randomly throughout the day either modified the phasing of the rhythm or suppressed it altogether (Delahunty et al. 1978). Because the hatchery fish were fed over the course of the day, plasma cortisol levels may not have fluctuated greatly.

The surge in plasma cortisol concentration during the spring is a result of enhanced interrenal activity during smoltification as shown by histological examination of interrenal tissue (Nishioka et al. 1982). The difference in magnitude of the surge is a reflection of interrenal cortisol production. Response of the interrenal to stress is dependent on rearing environment. Salonius and Iwama (1993) showed that wild and colonized coho smolts had significantly greater plasma cortisol concentrations than their hatchery counterparts when subjected to a 60-s handling stress. This difference may be due to a reduced sensitivity of the interrenal to ACTH. Regulation of cortisol secretion is mediated by negative feedback to the hypothalamus and pituitary (Fryer and Peter 1977) and cortisol secretion from the interrenal cells inhibits further interrenal response (Donaldson 1981).

We estimated clearance of cortisol from the plasma by the reduction in total [^{3}H] after injection of [^{3}H]cortisol. Brown et al. (1986) emphasize that clearance rates calculated using total radioactivity can underestimate the clearance rate of authentic cortisol by a factor of two to four, as cortisol is rapidly converted to cortisone in the plasma of the fish (Patino et al. 1985). Still, the half-life of radioactivity in the plasma can be used as a reasonable approximation of the clearance rate (Redding et al. 1984). It is possible that the rate of conversion of cortisol to cortisone may vary between the hatchery and wild fish. To what extent this would affect our results is not known.

Stress, transfer to sea water (Redding et al. 1984), and smolting (Patino et al. 1985) result in a decrease in the half-life of cortisol in the plasma of coho salmon. We also present evidence that the plasma cortisol half-life declines as the fish smolt. The cortisol half-life at each sampling interval over the spring did not differ significantly among the groups. As rearing environment affects cortisol dynamics, and the environment for the hatchery and wild fish was so vastly different, it is surprising that there is little difference among the groups, especially as other parameters measured differed significantly among the groups. It would appear that metabolism of cortisol is not as sensitive to stress as other parameters measured. In the present study, however, fish were captured and transferred to a aluminum trough and held for up to 24 h before determination of plasma cortisol half-life. It is possible that handling the fish may have affected this parameter. Redding et al. (1984) found that acute stress and acute treatment with cortisol did not significantly affect plasma clearance of cortisol, although chronic cortisol administration and stress caused an increase in cortisol clearance. The fish used in the study of Redding et al. (1984) were subjected to a severe stress for a 12 h prior to half-life determination. As Barton et al. (1986) showed that multiple stresses are cumulative, likely the procedure used by Redding et al. (1984) was more severe than treatment of fish in the present experiment. Despite a longer holding period in our study, we believe that little change in rate of plasma cortisol clearance occurred.

Hormone sensitivity is dependent on the number of hormone receptors in the target tissue (Danielsen and Stallcup 1985). In our study the gills of wild juvenile coho always possessed a greater number of corticosteroid receptors than those of hatchery fish, and would, therefore, be more sensitive to increases in cortisol concentration. Cortisol in turn will modify the concentration and affinity of corticosteroid receptors in gills of coho salmon (Maule and Schreck 1991; Shrimpton and Randall 1994). The decline in receptor number and affinity that we observed during the spring is similar to that seen with cortisol treatment. Thus, the seasonal change appears to be regulated by the cortisol surge associated with smolting. The change in CR, however, began to occur before plasma cortisol concentrations began to increase in the spring. Factors other than cortisol may also affect CR concentration and affinity. Although, growth hormone (GH) treatment will upregulate the concentration of gill CR (J.M. Shrimpton and R.H. Devlin, unpublished data), it is unlikely that there was an effect in the present study because the endogenous levels of GH in the plasma were seldom significantly different between the hatchery and wild coho (J.M. Shrimpton and R.M. Sweeting, unpublished data). The absolute difference in CR concentration between hatchery and wild coho, however, is likely due to the regulation of CR by cortisol. Shrimpton and Randall (1994) showed that stress will cause a downregulation of CR in the gills of juvenile coho salmon. The lower concentration of CR in the gills of hatchery fish may be a result of stresses associated with hatchery life, that are vastly different from the stresses experienced by wild fish (Donaldson 1981).

Owing to the greater concentrations of CR, the sensitivity of the gills to cortisol is greater in wild than hatchery smolts. This corresponds with a much greater responsiveness to cortisol in wild fish as indicated by a significantly greater increase in Na^+K^+ATPase activity during the spring and a strong positive relationship between Na^+K^+ATPase activity and plasma cortisol concentration (Fig. 9). The rise in Na^+K^+ATPase associated with cortisol increase is greatest in wild fish and lowest in hatchery fish from the bottom section of the earthen channel.

Na^+K^+ATPase activity and cortisol have been used extensively to quantify smolting. Wild fish in this study showed an increase in Na^+K^+ATPase activity, an increase in plasma cortisol, and a decline in CR affinity (increase in k_D). The significantly higher k_D seen in the 1992 data for the wild fish and their excellent performance in the 35‰ saltwater challenge test (Shrimpton et al. 1994) is evidence that k_D may be a useful parameter for evaluating smolting in coho salmon.

The role of cortisol in stimulation of smolting appears to be suppressed or inhibited in the fish reared in the hatchery.

The difference between the CR concentration in wild and hatchery 1 fish is similar to that between the hatchery 1 and hatchery 2 fish. The wild fish, however, show a significantly greater saltwater tolerance than both the hatchery groups of fish, which did not differ significantly. Therefore, it is likely that the difference in CR concentration in conjunction with the greater surge of cortisol in the wild smolts may account for the difference in saltwater tolerance of the hatchery and wild smolts. These findings have important implications for hatchery rearing practices in the production of salmon smolts.

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References

ALEXANDER, J.B., AND G.A. INGRAM. 1980. A comparison of five of the methods commonly used to measure protein concentrations in fish sera. J. Fish Biol. 16: 15–122.

BARTON, B.A., AND G.K. IWAMA. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1: 3–26.

BARTON, B.A., C.B. SCHRECK, R.D. EWING, A.R. HEMINGSEN, AND R. PATINO. 1985. Changes in plasma cortisol during stress and smoltification in coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 59: 468–471.

BARTON, B.A., C.B. SCHRECK, AND L.A. SIGISMONDI. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Trans. Am. Fish. Soc. 115: 245–251.

BENNETT, J.P. 1978. Methods in binding studies, p. 57–90. *In* H.I. Yamamura, S.J. Enna, and M.J. Kuhar [ed.] Neurotransmitter receptor binding. Raven Press, New York, N.Y.

BLACKMUN, G.J., B.V. LUKYN, W.E. MCLEAN, AND D. EWART. 1985. Quinsam watershed study: 1983. Can. MS Rep. Fish. Aquat. Sci. 1832: 65 p.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantiation of microgram quantities of protein using the principle of protein dye binding. Anal. Biochem. 72: 248–252.

BROWN, S.B., J.G. EALES, AND T.J. HARA. 1986. A protocol for estimation of cortisol plasma clearance in acid-exposed rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 62: 493–502.

BRY, C. 1982. Daily variations in plasma cortisol levels of individual female rainbow trout *Salmo gairdneri*: evidence for a post-feeding peak in well-adapted fish. Gen. Comp. Endocrinol. 48: 462–468.

DANIELSEN, M., AND M.R. STALLCUP. 1984. Down-regulation of glucocorticoid receptors in mouse lymphoma cell variants. Mol. Cell. Biol. 4: 449–453.

DELAHUNTY, G., J. OLCESE, M. PRACK, M.J. VODICNIK, C.B. SCHRECK, AND V. DE VLAMING. 1978. Diurnal variation in the physiology of the goldfish, *Carassius auratus*. J. Interdiscip. Cycle Res. 9: 73–88.

DONALDSON, E.M. 1981. Pituitary–interrenal axis as an indicator of stress in fish, p. 11–47. *In* A.D. Pickering [ed.] Stress and fish. Academic Press, London and New York.

FAGERLUND, U.H.M., J.R. MCBRIDE, B.S. DOSANJH, E.T. STONE, AND F.K. SANDERCOCK. 1983. Influence of culture density on juvenile coho salmon production and ocean survival. Can. Tech. Rep. Fish. Aquat. Sci. 1229: 27 p.

FRYER, J.N., AND R.E. PETER. 1977. Hypothalamic control of ACTH secretion in goldfish. III. Hypothalamic cortisol implant studies. Gen. Comp. Endocrinol. 33: 215–225.

HOAR, W.S. 1988. The physiology of smolting salmonids, p. 275–344. *In* W.S. Hoar and D.J. Randall [ed.] Fish Physiology Vol. 11B. Academic Press, New York, N.Y.

MADSEN, S.S. 1990. Cortisol treatment improves the development of hypoosmoregulatory ability in the euryhaline rainbow trout, *Salmo gairdneri*. Fish Physiol. Biochem. 8: 45–52.

MAULE, A.G., AND C.B. SCHRECK. 1990. Glucocorticoid receptors in leukocytes and gill of juvenile coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 77: 448–455.

MAULE, A.G., AND C.B. SCHRECK. 1991. Stress and cortisol treatment changed affinity and number of glucocorticoid receptors in leukocytes and gill of coho salmon. Gen. Comp. Endocrinol. 84: 83–93.

MCCORMICK, S.D., AND R.L. SAUNDERS. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. Am. Fish. Soc. Symp. 1: 211–229.

NISHIOKA, R.S., H.A. BERN, K.V. LAI, Y. NAGAHAMA, AND E.G. GRAU. 1982. Changes in the endocrine organs of coho salmon during normal and abnormal smoltification. An electron microscope study. Aquaculture. 28: 21–38.

NORMAND, M., AND C. FORTIER. 1970. Numerical versus analytical integration of hormonal disappearance data. Can. J. Physiol. Pharmacol. 48: 274–281.

PATINO, R., C.B. SCHRECK, J.L. BANKS, AND W.S. ZAUGG, 1986. Effects of rearing conditions on the developmental physiology of smolting coho salmon. Trans. Am. Fish. Soc. 115: 828–837.

PATINO, R., C.B. SCHRECK, AND J.M. REDDING. 1985. Clearance of plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol. 82A: 531–535.

PICKERING, A.D., AND T.G. POTTINGER. 1983. Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta* L. Gen. Comp. Endocrinol. 49: 232–239.

RANCE, T.A., B.I. BAKER, AND G. WEBLEY. 1982. Variations in plasma cortisol concentrations over a 24-h period in the rainbow trout *Salmo gairdneri*. Gen. Comp. Endocrinol. 48: 269–274.

REDDING, J.M., R. PATINO, AND C.B. SCHRECK. 1984. Clearance of corticosteroids in yearling coho salmon, *Oncorhynchus kisutch*, in freshwater and sea-water and after stress. Gen. Comp. Endocrinol. 54: 433–443.

RICHMAN, N.H., III, AND W.S. ZAUGG. 1987. Effects of cortisol and growth hormone on osmoregulation in pre- and desmoltified coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 65: 189–198.

SALONIUS, K., AND G.K. IWAMA. 1993. The effects of early rearing environment on stress response, immune function and disease resistance in coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon juveniles. Can. J. Fish. Aquat. Sci. 50: 759–766.

SCATCHARD, G. 1949. The attraction of protein for small molecules and ions. Ann. N.Y. Acad. Sci. 51: 661–672.

SCHRECK, C.B., R. PATINO, C.K. PRING, J.R. WINTON, AND J.W. HOLWAY. 1985. Effects of rearing density on indices of smoltification and performance of coho salmon, *Oncorhynchus kisutch*. Aquaculture 45: 345–358.

SHRIMPTON, J.M., AND D.J. RANDALL. 1994. Downregulation of corticosteroid receptors in the gills of coho salmon due to stress and cortisol treatment. Am. J. Physiol. 267: R432–R438.

SHRIMPTON, J.M., N.J. BERNIER, G.K. IWAMA, AND D.J. RANDALL. 1994. Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) before and after saltwater exposure. Can. J. Fish. Aquat. Sci. 51: 2170–2178.

SPECKER, J.L., AND C.B. SCHRECK. 1982. Changes in plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 46: 53–58.

STRANGE, R.J., C.B. SCHRECK, AND R.D. EWING. 1978. Cortisol concentrations in confined juvenile chinook salmon (*Oncorhynchus tshawytscha*). Trans. Am. Fish. Soc. 107: 812–819.

THORPE, J.E., AND M.G. MCCONWAY, M.S. MILES, AND J.S. MUIR. 1987. Diel and seasonal changes in resting plasma cortisol levels in juvenile Atlantic salmon, *Salmo salar* L. Gen. Comp. Endocrinol. 65: 19–22.

WAHLE, R.J., AND W.S. ZAUGG. 1982. Adult coho salmon recoveries and their Na^+K^+ATPase activity at release, Mar. Fish. Rev. 44: 11–13.

ZAUGG, W.S. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aquat. Sci. 39: 215–217.

ZAUGG, W.S. 1989. Migratory behaviour of underyearling *Oncorhynchus tshawytscha* and survival to adulthood as related to prerelease gill (Na^+K^+)-ATPase development. Aquaculture 82: 339–353.

The Effect of Short-duration Seawater Exposure on the Swimming Performance of Wild and Hatchery-reared Juvenile Coho Salmon (*Oncorhynchus kisutch*) during Smoltification

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The critical swimming velocity (U_{crit}) and haematology of wild and hatchery-reared coho salmon (*Oncorhynchus kisutch*) juveniles were examined in either fresh water or seawater following a 24-h seawater challenge, at the time of smoltification. In fresh water, wild smolts swam faster than hatchery-reared fish but this could largely be accounted for by scaling for body size. Transfer to seawater significantly elevated resting plasma [Na^+] and reduced subsequent U_{crit} in hatchery fish (by 12%) relative to that determined in fresh water but had no significant effect on resting plasma [Na^+] and U_{crit} in wild fish. Swimming the fish a second time in seawater after the initial 2-h exercise period resulted in a significant reduction in U_{crit} relative to that in fresh water in both wild fish (16%) and hatchery fish (a further 14%); this relatively greater impairment in U_{crit} in hatchery fish was due to a reduced hypo-osmoregulatory ability following seawater transfer that impairs conditions for muscle contractility and aerobic metabolism. Aerobic metabolism in seawater-exposed fish was affected in part through a reduction in haematocrit and an increase in plasma volume, reducing oxygen carrying capacity of the blood relative to conditions in fresh water.

On a mesuré la vitesse critique de nage (U_{crit}) et fait le bilan hématologique de juvéniles du saumon coho (*Oncorhynchus kisutch*) d'élevage et à l'état sauvage, dans l'eau douce ou dans l'eau de mer, après avoir challengé ces organismes par exposition à l'eau de mer pendant 24 h durant la période de la smoltification. Les saumoneaux à l'état sauvage placés dans l'eau douce nageaient plus vite que les saumoneaux d'élevage, mais cela pouvait être expliqué en bonne partie en introduisant des facteurs de correction pour les dimensions corporelles. Le transfert à l'eau de mer a provoqué une augmentation significative du [Na^+] plasmique chez les sujets au repos et à une réduction subséquente de la U_{crit} chez les sujets d'élevage (12%), relativement aux valeurs déterminées chez des sujets en eau douce, mais il n'avait pas d'effet significatif sur ces mêmes paramètres mesurés chez des sujets à l'état sauvage. Le fait de remettre les poissons une deuxième fois dans l'eau de mer après la période initiale d'exercice de deux heures, s'est traduit par une réduction significative de la U_{crit} relativement à ce qu'elle était chez les sujets à l'état sauvage (16 %) et les sujets d'élevage (une tranche additionnelle de 14 %) placés dans l'eau douce; cet abaissement relativement plus marqué de la U_{crit} chez les poissons d'élevage est attribuable à une perte de la capacité de régulation hypoosmotique après le transfert dans l'eau de mer, qui a pour effet de nuire à la contractilité musculaire et au métabolisme aérobie. Chez les sujets exposés à l'eau de mer, celui-ci a été en partie entravé par un abaissement de l'hématocrite et une augmentation volumique du plasma, avec pour effet de réduire la capacité de transport de l'oxygène dans le sang par rapport aux conditions que trouvaient les poissons dans l'eau douce.

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Artificial propagation of chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon in hatcheries has been used to repopulate river systems where existing stocks have been depleted and to supplement existing wild stocks to enhance both sport and commercial fisheries. The success of hatcheries and the role they should play in the future has recently been challenged (Hilborn 1992; Walters 1988).

Practices within many hatcheries have improved over the last several decades to maximize the genetic integrity of the artificially propagated salmonids by using escapement for brood stock rather than domesticated salmonids. The chinook used for brood stock in the Quinsam River hatchery in 1990 were determined to possess a high degree of variability at one locus relative to other coastal stocks (N.J. Bernier, Zoology Department, University of British Columbia, personal

communication). Thus, within reason, the genetic integrity of the returning spawners is conserved in the hatchery fish and differences between hatchery and wild coho salmon in this river system are largely phenotypic. Despite improvements in the hatchery production system, returns of hatchery fish are declining (Cross et al. 1991).

One of the most challenging transitions in the life history of a salmonid occurs when a smolt enters an estuary. This period of transition is preceded by a complete reorganization of osmoregulatory mechanisms and is thought to be a period of high mortality, particularly for those fish that have not completely smoltified (Parker 1962; Durkin 1982; Healy 1982). The success of a smolt in seawater is largely dependent on hypo-osmoregulatory ability. In the Quinsam River system Shrimpton et al. (1994) have demonstrated that at the time of smoltification there is a significant difference between wild and hatchery-reared coho salmon in their ability to hypo-osmoregulate following 24-h seawater exposure. This hypoosmoregulatory impairment in hatchery fish is correlated with reduced gill chloride cell numbers and enzyme activity (Na^+K^+ATPase and citrate synthase), compared with wild fish.

In coho salmon parr, there is a negative correlation between critical swimming velocity (U_{crit}) and plasma [Na^+] and [Cl^-] manipulated by seawater exposure (Brauner et al. 1992), but it is not known if this relationship exists in smolts. Physical performance capacity has been used as an index of salmonid viability (Bams 1964) because of its importance in functions such as predator avoidance, prey capture, migration, and competition. This study was conducted to determine if hypo-osmoregulatory ability affects swimming performance and haematology in wild and hatchery-reared coho salmon at the time of smoltification in the hopes of elucidating factors contributing to the reduced returns of hatchery-reared coho in British Columbia (Cross et al. 1991).

Materials and Methods

Fish Acquisition and Care

Hatchery-reared (weight = 21.2 ± 0.6 g, fork length = 13.7 ± 0.1 cm; mean ± SE) and wild fish (weight = 8.4 ± 0.3 g, fork length = 9.8 ± 0.1 cm) used in this study were obtained from the Quinsam river system in mid-May 1990. Wild fish were collected at an enumeration fence nightly as they migrated downstream. Hatchery-reared fish, propagated from adults returning to the Quinsam River system, were seined from the middle section of an earthen channel in the Quinsam River hatchery, and transported simultaneously with the wild fish to the University of British Columbia. Both groups were permitted to recover from transport for at least 1 wk prior to the experiments which were completed by the end of June 1990. Over the duration of this study, all fish were held in dechlorinated city water at 9°C and fed to satiation daily. All fish used in this study had the silvery appearance characteristic of smolts.

Experimental Procedure

Fish were starved for 72 h prior to experiments. Hatchery and wild fish were exposed for 24 h, separately, to both freshwater and seawater conditions (Marine mix, 29.2 ± 0.2‰) at 9.5 ± 0.9°C, in groups of 12 fish. The entire experiment consisted of six replications of each condition. Maximum fish densities did not exceed 3.0 $g \cdot L^{-1}$ as recommended by Blackburn and Clarke (1987). In each group of 12 fish, 3 were sampled to obtain resting parameters at the end of the 24-h exposure period, which was always between 09:00 and 10:00. These animals had been isolated from the rest of the group in a 25-L tank halfway through the exposure period so they could be anaesthetized as a means of minimizing sampling stresses (described below). The remaining nine fish were transferred into a 130-L Brett-type respirometer (described by Gehrke et al. 1990) filled with water at the salinity to which the fish had previously been exposed. The fish were introduced to the respirometer for 2 h at a swimming velocity of 18 $cm \cdot s^{-1}$, and water oxygen partial pressure of 153.8 ± 1.7 mmHg (1 mmHg = 133.3 Pa). Following the introductory period, the critical swimming velocity ($U_{crit}1$) was determined for each fish by increasing the water velocity by 1 body length per second ($BL \cdot s^{-1}$) every 30 min until the fish was fatigued. After each individual became fatigued, it was removed from the respirometer and three of the nine fish were killed immediately and sampled while the remaining six fish were placed in individual, thermostatically controlled recovery chambers for 2 h. Of the remaining six fish, three were killed and sampled after the 2-h recovery period while the other three were placed back in the respirometer for 2 h prior to a second U_{crit} determination ($U_{crit}2$). When these fish became fatigued, they were removed from the respirometer, killed, and sampled. Fish were deemed fatigued when they could no longer remove themselves from the rear screen of the respirometer despite repeated prodding.

Individual U_{crit} values were calculated by adding the last completed velocity to the product of the completed proportion of the increment in which the fish became fatigued and the magnitude of the increment that was 1 $BL \cdot s^{-1}$ in this study (Brett 1964). No correction for the solid blocking effect of the fish was made because the cross-sectional area of the fish did not exceed 5% of that of the swim tunnel. The entire experiment consisted of six replications of each condition: hatchery or wild fish exposed to either fresh water or seawater.

Sampling Procedure

Exhausted fish were netted, removed from the respirometer, and killed by concussion. Resting and recovering fish were anaesthetized by infusing a concentrated solution of tricaine methanesulfonate (MS 222) (final concentration 1 : 10 000) into the respective holding tank water to reduce handling stress associated with netting. Fish quickly lost equilibrium, were removed, and were killed by concussion. Blackburn and Clarke (1987) have demonstrated that anaesthetization prior to sampling does not affect plasma [Na^+]. In all fish, meristic measurements were recorded, the caudal peduncle was severed, and blood was collected in microhaematocrit tubes. Total haemoglobin concentration ([Hb]) of whole blood was measured using a Sigma total haemoglobin (525-A) assay kit and the relative absorbency measured at 540 nm in a Shimadzu UV-160 visible recording spectrophotometer. The haematocrit tubes were centrifuged at 11 500 rpm for 5 min in a Damon IEC MB microhaematocrit centrifuge and haematocrit (Hct) was measured in triplicate. Mean cell haemoglobin concentration (MCHC) was calculated using the formula: total [Hb] × 100/Hct.

Following blood removal, fish carcasses were frozen in liquid nitrogen and stored along with the haematocrit tubes containing plasma at −80°C for later analyses.

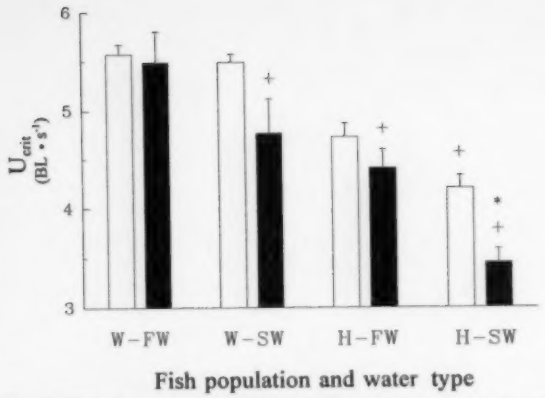

FIG. 1. First U_{crit} ($U_{crit}1$ open bar) and second U_{crit} ($U_{crit}2$; solid bar) for wild fish in fresh water (W–FW) and in seawater (W–SW), and hatchery-reared fish in fresh water (H–FW) and in seawater (H–SW). + indicates a significant difference from $U_{crit}1$ in fresh water for the respective fish population. * indicates a significant difference from $U_{crit}1$ in seawater.

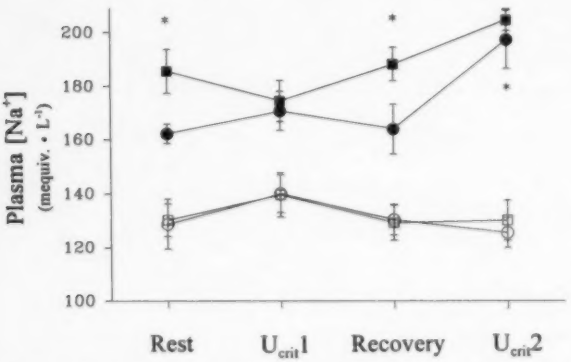

FIG. 2. Plasma [Na^+] in wild (circle) and hatchery-reared (square) coho salmon smolts in fresh water (open symbols) and following 24-h exposure to seawater (solid symbols) at rest, at fatigue following $U_{crit}1$, following 2-h recovery and at fatigue following $U_{crit}2$. * indicates a significant difference relative to the respective treatment in fresh water. For all data points $n = 6$, replicated in triplicate.

Analytical Techniques

Plasma in haematocrit tubes was thawed, expelled into an Eppendorf tube, and thoroughly mixed. To precipitate protein from the sample, 20 μL of plasma was combined with 20 μL of methanol, and 60 μL of distilled, deionized water in a 0.5-mL Eppendorf centrifuge tube. The contents of this tube were vortexed and centrifuged for 5 min in an Eppendorf centrifuge 3200. Twenty microlitres of the supernatant was then injected into a Shimadzu ion chromatograph (model HIC-6A) equipped with a Shodex Y521 cation column and 5 mM nitric acid mobile phase to determine plasma Na^+ concentration.

Plasma protein concentration was determined in plasma stored in haematocrit tubes by using the manual Biuret method (Alexander and Ingram 1980). Muscle moisture was determined on 0.5–1.0 g of dorsal epaxial muscle dried to constant weight at 60–70°C, which required up to 4 d.

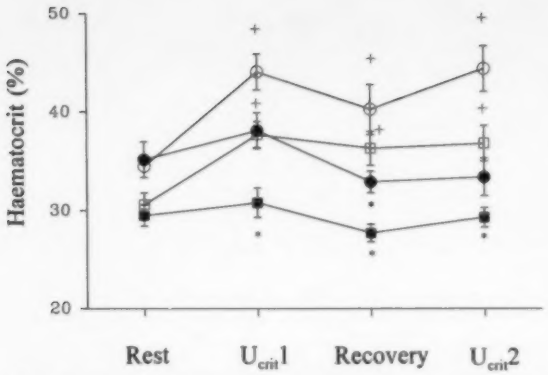

FIG. 3. Haematocrit in wild (circle) and hatchery-reared (square) coho salmon smolts in fresh water (open symbols) and following 24-h exposure to seawater (solid symbols) at rest, at fatigue following $U_{crit}1$, following 2-h recovery and at fatigue following $U_{crit}2$. * indicates a significant difference relative to the respective treatment in fresh water. + indicates a significant difference relative to resting conditions in the same water type. For all data points $n = 6$, replicated in triplicate.

Muscle moisture content was calculated by subtracting the final dry weight from the initial wet weight and dividing by the initial wet weight.

Statistics

Statistical differences among treatment means and exercise states were determined by a randomized complete block design two-way ANOVA followed by a post hoc Dunnett's multiple comparison test or a Tukey's test. Differences in U_{crit} were determined using a Student's t-test. A probability of 0.05 was chosen as the limit of statistical significance. All data reported are group means ± SE.

Results

The effect of seawater exposure and repeat swimming in both seawater and fresh water differed markedly between the groups of hatchery and wild fish (Fig. 1). In wild fish, the only significant reduction in U_{crit} was observed in fish forced to swim a second trial in seawater ($U_{crit}2$) (Fig. 1). In contrast, hatchery fish exhibited a significant reduction in $U_{crit}1$ upon transfer to seawater, and in both fresh water and seawater $U_{crit}2$ was significantly reduced relative to $U_{crit}1$ in the respective water types. In all treatments, U_{crit} was significantly lower for hatchery fish than wild fish; however, U_{crit} scales with body size and there were large differences in body size between the hatchery and wild fish.

In fresh water, there were no significant changes in plasma [Na^+] at any of the exercise states in either hatchery or wild fish (Fig. 2). During seawater exposure, plasma [Na^+] at rest was elevated relative to freshwater values in both groups but only significantly in hatchery fish. This was also the case after the recovery period. At $U_{crit}2$ plasma [Na^+] in both hatchery and wild fish swum in seawater was significantly elevated relative to that at $U_{crit}2$ in fresh water.

In both groups of fish in fresh water there was a statistically significant increase in Hct from resting values at $U_{crit}1$, recovery and $U_{crit}2$ (Fig. 3). In fish subjected to seawater exposure, there were no significant changes within each

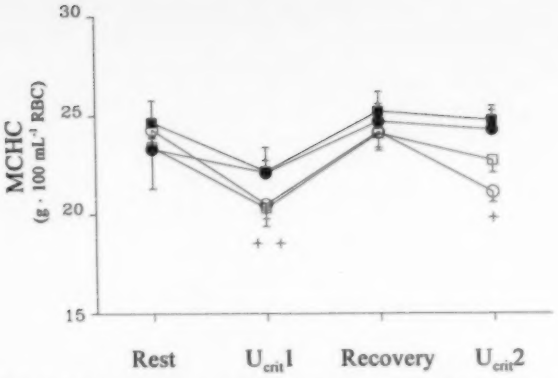

FIG. 4. Mean cell haemoglobin concentration (MCHC) (grams per 100 mL red blood cells) in wild (circle) and hatchery-reared (square) coho salmon smolts in fresh water (open symbols) and following 24-h exposure to seawater (solid symbols) at rest, at fatigue following $U_{crit}1$, following 2-h recovery and at fatigue following $U_{crit}2$. See Fig. 3 legend for further explanation.

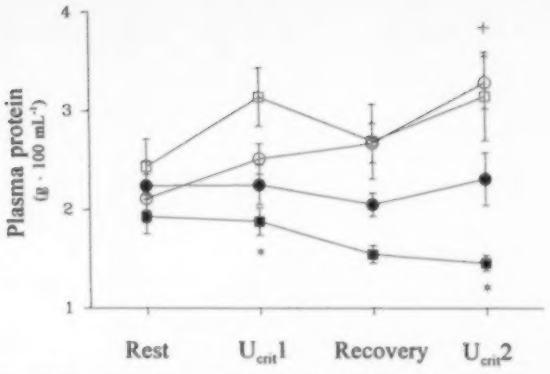

FIG. 5. Plasma protein concentration in wild (circle) and hatchery-reared (square) coho salmon smolts in fresh water (open symbols) and following 24-h exposure to seawater (solid symbols) at rest, at fatigue following $U_{crit}1$, following 2-h recovery and at fatigue following $U_{crit}2$. See Fig. 3 legend for further explanation.

group relative to resting Hct. In hatchery fish, Hct was significantly lower in seawater than in fresh water at $U_{crit}1$, recovery and $U_{crit}2$. In wild fish, the only significant difference in Hct in seawater relative to fresh water was observed during recovery.

In fresh water, there was a significant reduction in mean cell haemoglobin concentration (MCHC) relative to resting levels at $U_{crit}1$ in both groups of fish and at $U_{crit}2$ in the wild fish. MCHC did not differ significantly between fresh water and seawater in either hatchery or wild fish at any exercise state (Fig. 4), indicating that the relative reduction in Hct in seawater fish relative to freshwater fish, described above, was not due to shrinkage of red cells.

The only significant change in plasma protein concentration relative to resting levels was observed in wild fish in fresh water at $U_{crit}2$ (Fig. 5). The transfer to seawater resulted in a significant reduction in plasma protein concentration at $U_{crit}1$ and $U_{crit}2$ in hatchery-reared fish while no significant differences were observed in wild fish.

Muscle moisture content was influenced similarly by seawater exposure in the hatchery-reared and wild coho (Fig. 6). In both groups, there was a significantly lower muscle moisture at rest, recovery, and $U_{crit}2$ in fish exposed to seawater relative to those in fresh water. There were no significant changes with exercise within any of the groups.

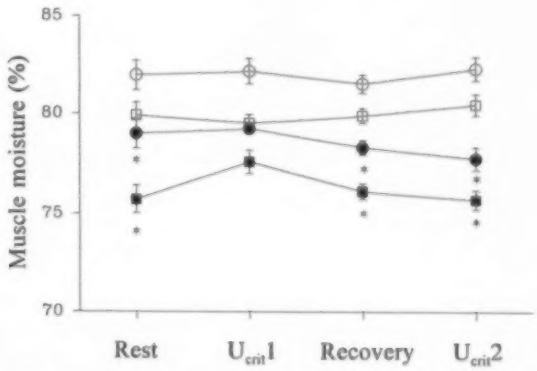

FIG. 6. Percent muscle moisture in wild (circle) and hatchery-reared (square) coho salmon smolts in fresh water (open symbols) and following 24-h exposure to seawater (solid symbols) at rest, at fatigue following $U_{crit}1$, following 2-h recovery and at fatigue following $U_{crit}2$. See Fig. 3 legend for further explanation.

Discussion

Swimming Performance

In all treatments, U_{crit} (expressed as $BL \cdot s^{-1}$) was significantly lower in hatchery fish than wild fish (Fig. 1) but much of this difference can be accounted for by the fact that the hatchery-reared fish are significantly larger than their wild counterparts. It is well established that there is a reduction in U_{crit} with an increase in body size when swimming velocity is expressed as a function of fish length ($BL \cdot s^{-1}$). When the relationship established for sockeye salmon (*O. nerka*) (Brett and Glass 1973) is applied to these data for coho salmon, there is no significant difference in freshwater $U_{crit}1$ between the two groups. Within each group of fish, however, U_{crit} is affected to a much greater degree in hatchery than in wild coho as a result of seawater exposure and repeated exercise in both freshwater and seawater. In hatchery fish exposed to seawater, the first U_{crit} ($U_{crit}1$) was significantly reduced relative to that in fresh water while there was no significant difference in $U_{crit}1$ in wild fish (Fig. 1). The relatively greater effect of seawater transfer on the swimming performance in hatchery-reared relative to wild salmonids has been observed by others (Smith 1987) and can be explained in part by the changes in plasma $[Na^+]$ associated with the 24-h seawater exposure prior to exercise. Following seawater exposure in hatchery fish, resting plasma $[Na^+]$ was elevated significantly (185.6 mequiv.$\cdot L^{-1}$) relative to freshwater values while in wild fish it was elevated insignificantly to 162.2 mequiv.$\cdot L^{-1}$. In hatchery-reared coho parr, an increase in resting plasma $[Na^+]$ to 173 mequiv$\cdot L^{-1}$ resulted in a significant reduction in subsequent U_{crit} while no effect on subsequent U_{crit} could be demonstrated if resting plasma $[Na^+]$ was approximately 160 mequiv.$\cdot L^{-1}$ or below (Brauner et al. 1992). These data suggest that the threshold for resting plasma $[Na^+]$ beyond which U_{crit} is

significantly reduced is similar for parr and juveniles at the time of smoltification. In addition, this threshold (between 162 and 186 mequiv.·L^{-1}) for exercise performance is similar to the value determined as the threshold for successful smolting (165–170 mequiv.·L^{-1} by Clarke and Blackburn 1977; Clarke and Shelbourn 1982; Blackburn and Clarke 1987) based upon subsequent growth rates measured in net pens and lends support to the use of physical performance indices, such as the U_{crit} test to determine the effect of environmental perturbations on the physical status of a given fish stock.

The U_{crit} in seawater is not solely dependent on plasma [Na^+] prior to exercise, because in fish forced to swim a second trial in seawater there was a significant reduction in U_{crit} ($U_{crit}2$) relative to $U_{crit}1$ in both hatchery and wild fish despite no change in preswim plasma [Na^+] within each group (Fig. 2). In general, the wild coho were capable of repeat exercise in fresh water with no effect on swimming performance, unlike their hatchery-reared counterparts, and there is a much greater effect of seawater transfer on U_{crit} in hatchery than in wild fish.

Physiological Basis for Impaired Swimming Performance

Although there is undoubtedly an anaerobic contribution to attaining U_{crit}, this test is predominantly dependent upon aerobic metabolism (Brett 1964). U_{crit} is reduced in rainbow trout (*O. mykiss*) by experimental anaemia (Jones 1971) and in chinook salmon, the functional haemoglobin concentration must be reduced by up to 50% before U_{crit} is significantly reduced (Brauner et al. 1993). Differences in U_{crit} between hatchery and wild coho may be explained by differences in conditions for aerobic metabolism (which must be quite large to have an effect) and muscle contractility. Generally, requirements for elevated aerobic metabolism at progressively increasing swimming velocities are met through an increase in oxygen uptake from the water and oxygen delivery to the active tissues. The latter is achieved through increases in cardiac output, oxygen extraction from venous blood, and oxygen-carrying capacity of the blood (Kiceniuk and Jones 1977). Most of these parameters were affected during seawater exposure and were impaired to a greater degree in hatchery-reared fish.

The differential impairment in swimming performance between hatchery and wild coho in seawater may be related to oxygen uptake at the gills. In resting rainbow trout 24 h after seawater transfer there is a 38% reduction in arterial oxygen content (Maxime et al. 1991), which may be in part due to lamellar dehydration causing a reduction in secondary lamellar surface area (Bath and Eddy 1979a). During short-term seawater exposure (less than 48 h) in resting Atlantic salmon (*Salmo salar*) there is a reduction in arterial pO_2 which is much greater in parr than smolts and is correlated with the degree of gill cell dehydration and seawater adaptability (Stagg et al. 1989; Eddy and Chang 1992). Gill cell dehydration was not measured in this study but there is no doubt that the seawater adaptability was greater in the wild than the hatchery-reared fish based upon resting plasma [Na^+] following 24-h seawater exposure, as well as gill Na^+K^+ATPase activity and cortisol receptor dynamics measured by Shrimpton et al. (1994) in the same fish population. Oxygen delivery may also have been reduced in hatchery fish due to the direct effect of elevated plasma ion concentrations on the heart. Cardiac output in toads has been demonstrated to decrease in proportion to the degree of hypernatremia (Hillman and Withers 1988). Prior to the U_{crit} determinations in seawater, plasma [Na^+] was significantly elevated relative to the respective condition in fresh water in the hatchery-reared but not the wild coho. If the relationship between plasma [Na^+] and cardiac output in fish is similar to that in toads, cardiac output would have been reduced in the hatchery fish, contributing to the impairment in swimming performance.

Oxygen-carrying capacity of the blood was affected differently in hatchery and wild fish during exercise in both fresh water and seawater. In fresh water, there was a characteristic increase in haematocrit at U_{crit} in both wild and hatchery-reared coho (Fig. 3). In fresh water, there was a trend toward an increase in plasma protein concentration that was significant in wild fish at $U_{crit}2$ (Fig. 5) indicating that the increase in haematocrit in fresh water was in part due to a decrease in plasma volume. No changes in muscle moisture were observed between rest and exercise in this study and, therefore, the reduction in plasma volume was not due to fluid shifts into the muscle. The increase in Hct in fish in fresh water was also due to an increase in mean cell volume, as indicated by the reduction in MCHC (Fig 4), probably because of the release of catecholamines (Baroin et al. 1984; Nikinmaa et al. 1984; Primmett et al. 1986; Cossins 1989; Nikinmaa 1990).

In contrast with the situation in fresh water, there was no significant change in Hct in either group of seawater-exposed fish. Hct was relatively lower at all activity levels in seawater relative to that in fresh water (Fig. 3). A reduction in Hct following seawater exposure in resting salmonids has been attributed to red cell shrinkage, caused by increased concentrations of plasma ions (Blackburn and Clarke 1987); however, in this study there was either a nonsignificant decrease in MCHC or a trend toward swelling (Fig. 4). The effect of seawater exposure on MCHC was the same in both groups of fish, which does not explain the relative differences in swimming performance between the two groups of fish. The relatively lower Hct in fish swum in seawater as compared with fresh water must have been either due to a reduced splenic contribution of red cells to the primary circulation or a lack of a decrease in the volume of the plasma compartment that was observed in fresh water. The former seems unlikely given the increase in oxygen demand associated with exercise. Further, plasma protein concentration was significantly lower in fish swum in seawater relative to fresh water, indicating that the plasma compartment was not decreasing as it was in animals exposed to fresh water. The maintenance of blood volume during exercise in sea water may be due to an increase in drinking rate following seawater transfer (in rainbow trout: Bath and Eddy 1979b; Eddy and Bath 1979; in Atlantic salmon smolts: Eddy and Chang 1992) and a reduction in urination rate (in rainbow trout: Bath and Eddy 1979a). In addition, the elevation in plasma ion concentrations may oppose fluid shifts from the plasma seen in fresh water. The lack of haemoconcentration during seawater exposure would reduce the oxygen content of the blood and may have been partly responsible for the reduction in U_{crit} seen in seawater. As with U_{crit}, the differences in Hct and plasma protein concentration between fresh-water- and seawater-exposed fish was more pronounced in the hatchery group.

Oxygen content of the blood may also be affected during seawater exposure independent of changes in Hct. Although plasma [Cl^-] was not measured in this study, it likely increased in accordance with plasma [Na^+] during seawater exposure as it does in coho parr (Brauner et al. 1992). In rainbow trout 24 h after seawater transfer, there is a reduction in arterial oxygen content due to a decrease in haemoglobin–oxygen affinity, partly because of an increase in red cell chloride concentration (Maxime et al. 1991). Thus, if there is a reduction in haemoglobin–oxygen affinity associated with elevated red cell [Cl^-] in coho salmon, it would be more severe in hatchery than wild fish.

The impairment in swimming performance in seawater may also lie at the level of the muscle due to the direct effect of elevated plasma ion concentrations on contractility (Homsher et al. 1974) or the neuromuscular apparatus (Houston 1959). Again, this effect on contractility would be expected to be greater in hatchery than in wild fish based on hypo-osmoregulatory differences between the two groups. The degree of muscle dehydration associated with seawater exposure is significantly reduced relative to conditions in fresh water in both hatchery and wild fish (Fig. 6). Muscle dehydration, therefore, cannot explain the differential impairment in U_{crit} in seawater between the two groups but may contribute to the general impairment in swimming performance seen in seawater.

Swimming performance may be limited through elevations in plasma ion concentrations and subsequent changes in the oxygen-carrying capacity of the blood ultimately affecting oxygen delivery to the muscles and muscle contractility. Although the physiological changes associated with seawater exposure were similar in the two groups of fish, they were generally more severe in the hatchery than in the wild fish.

Conclusion

Interpretation of results of the U_{crit} test is limited because this test cannot possibly take into account variables crucial for survival, such as behaviour. In addition, further development of the smolts may have occurred under natural conditions and may have been impaired by the conditions of this study. However, it is also possible that the results of this test may underestimate the differences that exist between the two groups because the fish were only forced to exercise twice, whereas survival is dependent upon continuous exercise. The salinity to which the fish were exposed (29‰) is similar to that in an estuary, increasing the relevance of the results obtained in the lab to the natural environment. Thus, in these experimental conditions the results of the U_{crit} test provide a relative index by which the physical status of hatchery-reared and wild fish can be quantified and compared.

There is a marked impairment in swimming performance in hatchery fish upon transfer to seawater and under forced repeat swimming in both fresh water and seawater that is virtually absent in wild fish. This impairment is largely a result of the difference in hypo-osmoregulatory ability between these two groups of fish. The Quinsam river hatchery uses returning spawners from a large run as coho brood stock. In chinook salmon the Quinsam hatchery brood stock for 1990 displayed a high degree of variability at one locus relative to other coastal chinook stocks (N.J. Bernier, Zoology Department, University of British Columbia, personal communication). Assuming that the hatchery and wild groups of fish are genotypically similar, differences in their ability to hypo-osmoregulate are phenotypic. This is further supported by the greater survival of smolt to adult in colonized fish (10–20%, 1978–82) relative to hatchery-reared fish (5–8%, 1978–82) in the Quinsam River system (W.E. McLean, Quinam River Hatchery, personal communication). The colonized fish are translocated from the hatchery to the upper watershed 8 mo before smolting and thus spend less time in the hatchery. Thus differences in rearing conditions such as water temperature, diet, rearing density, and water quality (see Shrimpton et al. 1994 for greater detail) may be important determinants of smolt survival subsequent to release from a hatchery. The results obtained in these fish may differ from those obtained in other river systems but the important point is that artificial propagation of salmonids similar in quality to wild fish is not as easy as once thought. Thus, we may not simply be able to compensate for human intervention in a river system by replacing a wild stock once it has been lost.

Acknowledgements

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References

ALEXANDER, J.B., AND G.A. INGRAM. 1980. A comparison of the methods commonly used to measure protein concentrations in fish sera. J. Fish Biol. 16: 115–122.

BAMS, R.A. 1964. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. J. Fish. Res. Board Can. 24(5): 1117–1153.

BAROIN, A., F. GARCIA-ROMEU, T. LAMARRE, AND R. MOTAIS. 1984. Hormone-induced co-transport with specific pharmacological properties in erythrocytes of rainbow trout, *Salmo gairdneri*. J. Physiol. 350: 137–157.

BATH, R.N., AND F.B. EDDY. 1979a. Ionic and respiratory regulation in rainbow trout during rapid transfer to seawater. J. Comp. Physiol. 134: 351–357.

BATH, R.N., AND F.B. EDDY. 1979b. Salt and water balance in rainbow trout (*Salmo gairdneri*) rapidly transferred from fresh water to sea water. J. Exp. Biol. 83: 193–202.

BLACKBURN, J., AND W.C. CLARKE. 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1515: 35 p.

BRAUNER, C.J., J.M. SHRIMPTON, AND D.J. RANDALL. 1992. The effect of short duration seawater exposure on plasma ion concentrations and swimming performance in coho salmon (*Oncorhynchus kisutch*) parr. Can. J. Fish. Aquat. Sci. 49: 2399–2405.

BRAUNER, C.J., A.L. VAL, AND D.J. RANDALL. 1993. The effect of graded methaemoglobin levels on the swimming performance in chinook salmon (*Oncorhynchus tshawytscha*). J. Exp. Biol. 185: 121–135.

BRETT, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21(5): 1183–1226.

BRETT, J.R., AND N.R. GLASS. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. J. Fish. Res. Board Can. 30: 379–387.

CLARKE, W.C., AND J. BLACKBURN. 1977. A seawater challenge test to measure smolting of juvenile salmon. Can. Fish. Mar. Serv. Tech. Rep. 705: 11 p.

CLARKE, W.C., AND J.E. SHELBOURN. 1982. Growth and smolting of underyearling coho salmon in relation to photoperiod and temperature, p. 200–216. *In* Proceedings of the North Pacific Aquaculture Symposium, Aug. 1980. Anchorage, Alaska. Alaska Sea Grant Rep. 82-2.

COSSINS, A. 1989. Intracellular pH regulation by fish red cells. Nature (Lond.) 340(6): 20–21.

CROSS, C.L., LAPI, L., AND E.A. PERRY. 1991. Production of chinook and coho salmon from British Columbia hatcheries, 1971 through 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1816: 48 p.

DURKIN, J.T. 1982. Migration characteristics of coho salmon (*Oncorhynchus kisutch*) smolts in the Columbia River and its estuary, p. 365–376. *In* V.S. Kennedy [ed.] Estuarine comparisons. Academic Press, New York, N.Y.

EDDY, F.B., AND R.N. BATH. 1979. Ionic regulation in rainbow trout (*Salmo gairdneri*) adapted to fresh water and dilute sea water. J. Exp. Biol. 83: 181–192.

EDDY, F.B., AND Y.J. CHANG. 1992. Effects of salinity in relation to migration and development in fish, p. 35–43. *In* J. Eduardo and P.W. Bicudo [eds.] The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life: Proceedings of a Meeting. Sept. 10–15, 1991, Sao Sebastio, Sao Paulo, Brazil. CRC Press, Inc., Boca Raton, Fla.

GEHRKE, P.C., L.E. FIDLER, D.C. MENSE, AND D.J. RANDALL. 1990. A respirometer with controlled water quality and computerized data acquisition for experiments with swimming fish. Fish Physiol. Biochem. 8(1): 61–67.

HEALY, M.C. 1982. Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can. J. Fish. Aquat. Sci. 39: 952–957.

HILBORN, R. 1992. Hatcheries and the future of salmon in the northwest. Fisheries (Bethesda) 17(1): 5–8.

HILLMAN, S.S., AND P.C. WITHERS. 1988. The hemodynamic consequences of hemorrhage and hypernatremia in two amphibians. J. Comp. Physiol. B Metab. Transp. Funct. 157: 807–812.

HOMSHER, E., F.N. BRIGGS, AND R.M. WISE. 1974. Effects of hypertonicity on resting and contracting frog skeletal muscles. Am. J. Physiol. 226(4): 855–863.

HOUSTON, A.H. 1959. Locomotor performance of chum salmon fry (*Oncorhynchus keta*) during osmoregulatory adaptations to sea water. Can. J. Zool. 37: 591–605.

JONES, D.R. 1971. The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 55: 541–551.

KICENIUK, J.W., AND D.R. JONES. 1977. The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. J. Exp. Biol. 69: 247–260.

MAXIME, V., J.C. PENNEC, AND C. PEYRAUD. 1991. Effects of direct transfer from freshwater to seawater on respiratory and circulatory variables and acid–base status in rainbow trout. J. Comp. Physiol. B Metab. Transp. Funct. 161: 557–568.

NIKINMAA, M. 1990. Vertebrate red cells. Zoophysiology 28. Springer-Verlag, Berlin.

NIKINMAA, M., J.J. CECH, AND M. MCENROE. 1984. Blood oxygen transport in stressed striped bass (*Morone saxatalis*): role of beta-adrenergic responses. J. Comp. Physiol. B Metab. Transp. Funct. 154: 365–369.

PARKER, R.R. 1962. Estimations of ocean mortality rates for Pacific salmon (*Oncorhynchus*). J. Fish. Res. Board Can. 19: 561–589.

PRIMMETT, D.R.N., D.J. RANDALL, M. MAZEAUD, AND R.G. BOUTILIER. 1986. The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*) during exercise. J. Exp. Biol. 122: 139–148.

SHRIMPTON, J.M., N.J. BERNIER, G.K. IWAMA, AND D.J. RANDALL. 1994. Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) before and after saltwater exposure. Can. J. Fish. Aquat. Sci. 51: 2170–2178.

SMITH, L. 1987. Health measurements, p. 97–108. *In* Improving hatchery effectiveness as related to smoltification. U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Project 84-52.

STAGG, R.M., C. TALBOT, F.B. EDDY, AND M. WILLIAMS. 1989. Seasonal variations in osmoregulatory and respiratory responses to sea water exposure of juvenile Atlantic salmon (*Salmo salar*) maintained in fresh water. Aquaculture, 82: 219–228.

WALTERS, C. 1988. Mixed-stock fisheries and the sustainability of enhancement production for chinook and coho salmon, p. 109–115. *In* W.J. McNeil [ed.] Salmon production, management, and allocation. Biological, economic, and policy issues. Oregon State University Press, Corvallis, Oreg.

Incomplete Recovery in a Coastal Fish Community Exposed to Effluent from a Modernized Swedish Bleached Kraft Mill

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Between 1982 and 1987, many abnormalities were reported in a Baltic coastal fish community exposed to bleached kraft mill effluent. Perch (*Perca fluviatilis*) was selected as a sentinel species. Local recruitment was significantly reduced owing to high larval mortality associated with gonad malfunctions and low embryo quality. Adult abundance was low near the mill outfall and the community was poor in species. After 1984, the toxicity of the effluent was reduced by substituting chlorine dioxide for molecular chlorine which resulted in lower amounts of organochlorine waste, while other effluent variables remained relatively unchanged. In 1992 the use of chlorine was discontinued. A follow-up study on fish showed that many previously documented abnormalities were absent, e.g., symptoms of external disease, lower rate of sexual maturation, and reduced relative gonad size. The number of species had increased, and the fish community resembled a more normal species assemblage. However, perch embryos produced in the effluent area remained smaller than those in a local reference area and larval survival was low. Consequently, although reduced organochlorine waste discharge had resulted in environmental improvement, recovery in the perch population was incomplete.

Entre 1982 et 1987, on a rapporté beaucoup d'anomalies chez une communauté de poissons littoraux de la mer Baltique qui était exposée aux effluents d'une fabrique de pâte kraft blanchie. La perche d'Europe (*Perca fluviatilis*) a été choisie comme espèce témoin. Le recrutement local était significativement abaissé par suite de la mortalité élevée des larves associée au dysfonctionnement des gonades et du mauvais état des embryons. À proximité de l'exutoire de la fabrique, il y avait peu d'adultes et la communauté comptait peu d'espèces. Depuis 1984, la toxicité de l'effluent a été atténuée par substitution du bioxyde de chlore au chlore moléculaire; cela a eu pour effet d'abaisser la quantité de résidus organochlorés tandis que les autres variables des effluents sont restées relativement les mêmes. En 1992, la fabrique a cessé d'utiliser du chlore. Le suivi a montré que bon nombre des anomalies antérieurement observées étaient disparues; c'étaient notamment les symptômes de troubles en surface des organismes, le taux réduit de maturation sexuelle et la taille relative réduite des gonades. Le nombre d'espèces avait augmenté et les groupes d'espèces ressemblaient davantage à une communauté normale. Cependant, les embryons de perche produits dans le secteur contaminé par les effluents, restaient plus petits que ceux de secteurs témoins de la région, et la survie des larves était restée mauvaise. Par conséquent, même si la réduction des rejets de résidus organochlorés a amélioré les conditions du milieu, il demeure que la population de perches ne s'est pas complètement rétablie.

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A Swedish research programme was started in 1982 with the primary objective of evaluating the environmental risks associated with the use of molecular chlorine in pulp bleaching. The coastal area receiving wastewater from the Norrsundet pulp mill was selected for the studies. It was evident that the resident fish community in this area was exposed to toxic substances, and effects could be seen at most organizational levels, from cell to community (Södergren 1989).

The general nature of these observations could be verified at other Swedish (Sandström et al. 1991a, 1991b; Förlin et al. 1991) as well as Canadian mills (Hodson et al. 1991; McMaster 1991; Munkittrick et al. 1992), contributing to the evidence suggesting that bleached pulp production was the cause of the effects observed. However, some authors stressed that a strict cause–effect analysis was not possible on the basis of these field studies. Many different substances may act in a pulp mill effluent area, causing a range of reactions from eutrophication to chronic toxicity, and it was not known to what extent the toxic reactions were really related to chlorinated substances.

The debate, however, concentrated on chlorine bleaching, and the Swedish pulping industry responded immediately by substituting other chemicals, mainly chlorine dioxide, for molecular chlorine resulting in smaller amounts of chlorinated organics leaving the modernized plants. The effluent toxicity was also reduced by the lower degree of chlorination of the organic molecules when using chlorine dioxide. Although effluents from pulp mills have changed substantially and much money has been invested in the process, little is yet known of the environmental improvements actually obtained.

Chlorine dioxide was also successfully introduced at the Norrsundet mill, reducing adsorbable, organically bound halogen (AOX) discharges considerably. As other effluent characteristics remained almost unchanged and, as there was a considerable data set available for retrospective

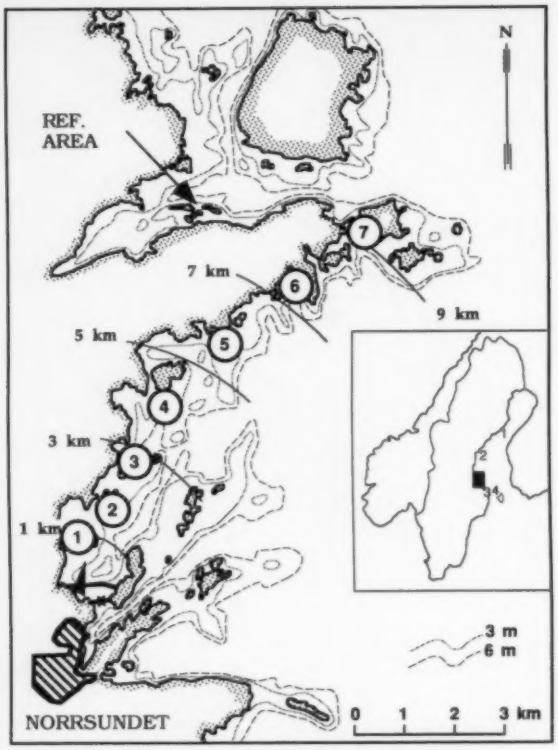

FIG. 1. The Norrsundet effluent area with local reference and sampling sites (circled nos. 1–7) indicated. *X* = water intake for effluent mixing. Arrowhead is discharge point of aerated mixed effluent and water. Numbers 1–4 on inset are distant reference sites.

comparisons, this mill provided a good opportunity to study environmental effects of the general trend in pulp bleaching. A proposal to start follow-up studies on fish was accepted by the company owning the mill, and a sampling programme identical to the one reported by Sandström et al. (1988) and Karås et al. (1991) was financed. This paper includes studies on adult gonad development, spawning, roe and embryo development, larval abundances, adult abundances, and community structure. The reproduction and recruitment studies were made on perch (*Perca fluviatilis*), a common resident species of these waters.

Material and Methods

The Norrsundet pulp mill is situated on the coast of the Bothnian Sea (northern Baltic; Fig. 1). Bleached pulp is produced, mainly from softwood, and the annual production is about 240 000 t. The effluent characteristics are measured at the mill according to standardized protocols under the supervision of the county authorities. Chemical oxygen demand (COD) and suspended solids in the effluents are measured daily. Before autumn 1987, discharges of AOX were calculated from the daily use of molecular chlorine and from more irregular total organic chlorine (TOCl) analyses. During 1987–1990, AOX was measured daily, followed by weekly analyses of pooled samples during 1991–1992. Until 1992, secondary treatment was restricted to a removal of fibers, etc., in a sedimentation system. Aerated lagoons, prepared for active sludge treatment, were installed in 1992, but were not used until the autumn, when the present research had been completed. The mill effluents were diluted about 40 times before being discharged by mixing with seawater pumped from a nearby bay (Fig. 1).

During 1983, about 12 t of chlorine were used for bleaching, resulting in AOX levels of about 6 kg/t air-dried pulp (ADP) (Fig. 2). The use of molecular chlorine was reduced stepwise and totally stopped in April 1992. After the chlorine dioxide substitution was completed, AOX discharges were about 0.5 kg/t ADP. During the later part of the study period (1987–1992) other effluent variables, such as COD and suspended solids, changed little (Fig. 2).

The sampling stations for test fishing and perch reproduction studies were situated along the shore in shallow water (3–5 m), roughly following the effluent concentration gradient (Fig. 1). A local reference area for spawning and larvae studies was selected in a nearby bay north of the effluent area. The current on the Swedish side of the Baltic trends generally southwards, and thus the effluents will only reach the reference area in the presence of strong southeast winds. The sampling stations corresponded to those visited in previously reported studies (Neuman and Karås 1988; Sandström et al. 1988; Sandström and Thoresson 1988; Karås et al. 1991). Reference samplings of perch for maturation rate studies in 1984 and 1985, however, were made in an area about 100 km south of Norrsundet. As there were no significant differences between maturation rates at this site and at the local reference stations (9 and 14 km from the mill; Sandström et al. 1988), this distant reference area was not used in the repeated studies.

Additional data for species distributions and abundance comparisons were provided from four coastal reference areas in the northern, central, and southern Bothnian Sea (Fig. 1). Long-term monitoring has been running in area 1 since 1989 and in area 4 since 1983. In reference areas 2 and 3 data were available only for 1 y, 1991.

The Baltic is brackish, and the salinity in Bothnian Sea coastal waters is about 0.4%. The tidal influence is insignificant. In the effluent area, effects of wind-driven or other currents are generally small, especially in its inner parts, owing to the topography and the shallow water. Sampling sites 1 and 2 are protected from the main effluent stream by a sill about 1 m deep. As there are no major freshwater inflows, salinity varies little during the year.

All sampling and analyses were conducted using techniques reported in earlier studies (Sandström et al. 1988; Karås et al. 1991) to allow direct comparison of data from 1982 to 1987 with the new information reported in this study from 1989 to 1992.

Adult fish were sampled for gonad analyses in autumn 1989 and 1990 at stations 1 and 2 and at the local reference site. The collections were made simultaneously at all stations during a period of 2 or 3 subsequent nights. Total length (mm), total weight (0.1 g), somatic weight (0.1 g; alimentary tract and gonad removed), and gonad weight (0.1 g) were measured, and sex and sex stage were determined. The condition factor (CF) was calculated as somatic weight in grams $\times$ 100 / (total length in centimetres)^3. The gonadosomatic index (GSI) was expressed as gonad weight calculated as a percentage of total body weight. A condition-independent measure of relative gonad size was calculated by relating

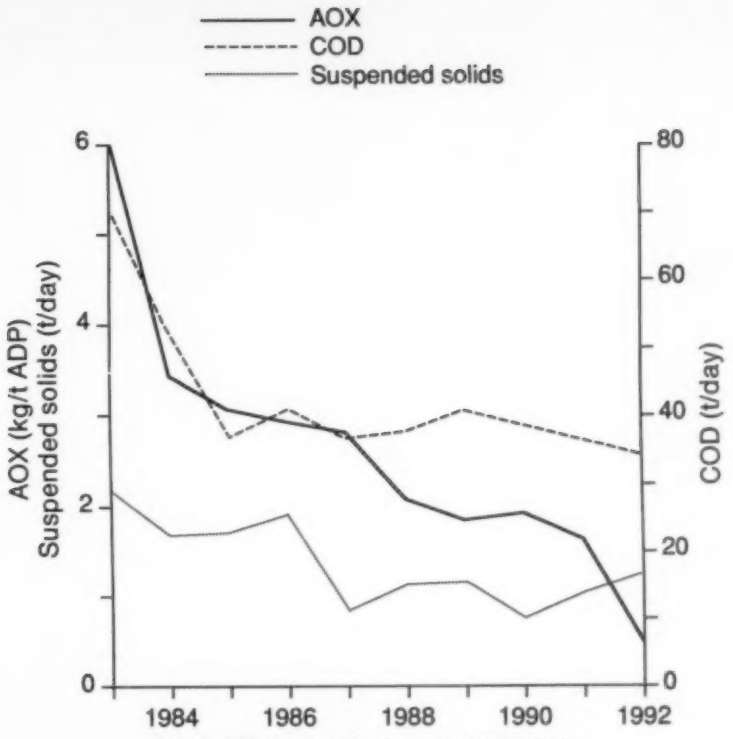

FIG. 2. Effluent data for the period 1983–1992.

gonad weight to total length. To investigate the possibility of delayed sexual maturation, fish large enough to be potentially mature were selected. As established in studies at reference areas, a lower limit for maturity was set at 170 mm for females and at 150 mm for males (Sandström et al. 1988). Fish over these sizes were divided into developing and undeveloped categories on the basis of gonad size.

Spawning was studied in 1992 using artificial substrates (Karås et al. 1991) placed at stations 1 and 2 and at the local reference site (Fig. 1). The stations were visited once a week from May 14 to June 17. Perch spawn by attaching one single string of roe on aquatic plants, etc. By counting the number of roe strings attached to the artificial substrates throughout the spawning season, spawning intensity was estimated in the different areas. Water temperature was measured when visiting the stations. Close to the time of hatching, roe strings were preserved in formaldehyde for egg and embryo analyses in the laboratory. Ten strings from each of stations 1 and 2 and 9 from the reference site were checked for roe mortality by examining about 200 eggs per string. Two hundred embryos per string were separated from the egg under a stereomicroscope, using thin needles, to check for morphological malformations. Total body length (mm) was measured on 25 randomly selected embryos per string. Weight (dry weight, mg) with an without yolk sac was measured on pooled samples of 100 embryos per string.

After hatching started, pelagic larvae were sampled with a twin-coupled Bongo net (mesh size 0.3 mm, with flow meter), towed at 0.5–1 m depth for about 100 m (filtered volume 30–60 m^3) during daytime at stations 1 and 2 and at the local reference site (Fig. 1). Five tows per station were made on each sampling date. The tows were made on June 2, 6, and 16. By comparing results from different samplings, the period of maximal hatching was established. Abundance data for newly hatched (<9 mm) larvae from sampling sites at the date of maximal hatching were compared graphically. A similar analysis was performed on the previously reported results from 1987 (Karås et al. 1991). A nonparametric method (Mann–Whitney *U*-test) was used for statistical comparisons, as the abundances were not normally distributed.

During August 1992, test fishing for species diversity and relative abundance comparisons was conducted at seven stations (Fig. 1), using 3-m-high and 35-m-long multi-mesh-size gillnets (Neuman and Karås 1988; Karås et al. 1991; Thoresson 1993). Four nets were set at each station. The water depth varied between 3 and 6 m at the sampling stations. The fishing was repeated six times during a 2-wk period. When recording the catches of different species, visual inspections were made on all specimens for external injuries or morphological malformations such as the previously abundant fin erosion (Lindesjöö and Thulin 1990). Test fishing in distant reference areas 1, 2, 3, and 4 (Fig. 1) was performed according to the same guidelines (Thoresson 1993). Water depth and exposure to the open sea were similar to those in the effluent area.

When comparing yearly abundance distributions along the Norrsundet effluent gradient, the between-year differences in mean catch per unit effort (depending, for example, upon temperature variations) were eliminated by a normalization technique (Neuman and Karås 1988). The mean catch per unit effort calculated for all stations in a single year was set at 1, and the catches at different stations were related

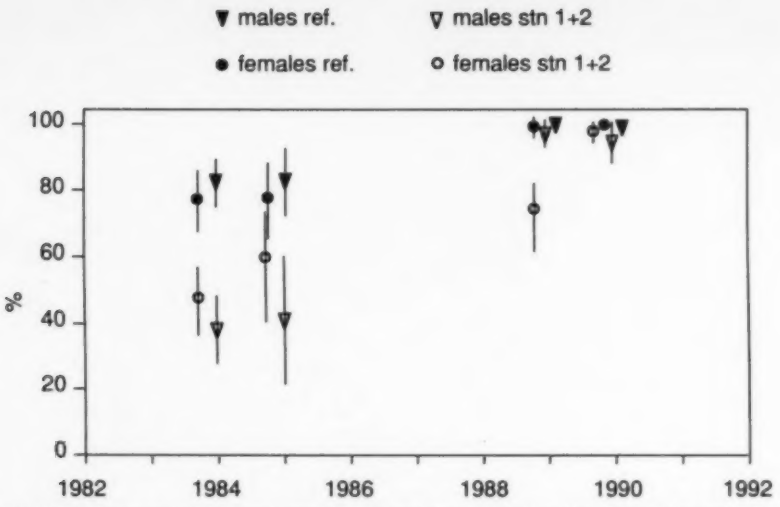

FIG. 3. Maturity rate (%) in perch sampled at stations 1 and 2 at the Norrsundet pulp mill in 1984, 1985, 1989, and 1990. Reference samples were collected in Öregrundsgrepen, about 100 km south of the effluent area, during 1984–1985. In 1989–1990 the local reference area was used for the comparisons. A confidence limit of 95% is given when 100% maturity was not reached in the samples.

TABLE 1. Gonadosomatic index (GSI), condition factor (CF), and the relation gonad weight/total length (GW/L) in perch from station 1 in the Norrsundet effluent area in 1989 and 1990 compared with the local reference site. Means and sample sizes (*N*).

Year	Sex	Station	*N*	GSI	CF	GW/L
1989	Female	1	49	4.6*	1.18*	3.84
		Reference	76	5.5	1.10	3.71
	Male	1	41	6.5	1.21*	4.85
		Reference	23	7.0	1.11	4.74
1990	Female	1	39	8.4*	1.12*	6.62
		Reference	61	9.6	1.07	6.33
	Male	1	20	5.9	1.09	3.73
		Reference	26	6.1	1.05	3.46

*Significantly different ($p < 0.05$) from the reference site (*t*-test).

to this standard. For example, when the mean catch at a station was twice as high as the mean for all stations, the normalized catch was expressed graphically as 2. The Kruskal–Wallis test (Kruskal and Wallis 1952) was used for comparisons of stations, accompanied by a nonparametric method analogous to Neuman–Keuls' test (Zar 1974). Differences were considered significant at the 5% level.

Results

Gonad Development

In previous studies (Sandström et al. 1988) the ratio of mature/immature perch during autumn was considerably lower (χ^2 test, $p < 0.05$) close to the mill than at station 7, as well as at the more distant reference site used in these studies (Fig. 3), indicating either delayed sexual maturation or inhibited gonad recrudescense after spawning. A significant difference was still evident in females, but not in males, during the 1989 sampling. When the study was repeated in 1990, effects on sexual maturation could no longer be demonstrated and the maturation rate was close to 100% for both sexes (Fig. 3).

Relative gonad size was examined in fish identified as mature and preparing for spawning next spring by analysing GSI and gonad weight – total body length relationships (Table 1). GSI calculations can illustrate variations in relative gonad size, if there are no differences among samples in somatic body condition. When the CF-values deviate, gonad weight – body length relations will provide more correct information. In 1983–1985, GSI values differed significantly between stations for both sexes, but because the CF-values were higher at stations close to the mill outfall, a gonad weight – total length regression analysis was made. Both females and males from stations 1–4 had significantly smaller gonads compared with reference fish of the same body length (Sandström et al. 1988). When the sampling was repeated in 1989, there were no significant differences in male GSI. Females from stations 1 and 2 had lower GSI than the reference fish, a difference also evident in 1990 (*t*-test, $p < 0.05$). However, as the CF-values still differed significantly, with higher condition at the exposed site (Table 1), the relation between gonad weight and total body length was calculated. No significant differences could be detected (Table 1), and the lower observed GSI of females in 1989 and 1990 evidently was an effect of the fish having a higher condition factor in the effluent exposed area, not an effect of reduced gonad size. The differences in gonad development in 1983–1985 consequently could no longer be demonstrated.

Spawning, Egg, and Embryo Studies

The number of attached roe strings on the spawning substrates did not differ significantly among stations (ANOVA and *F*-test, $p < 0.05$; Fig. 4) and corresponded with the

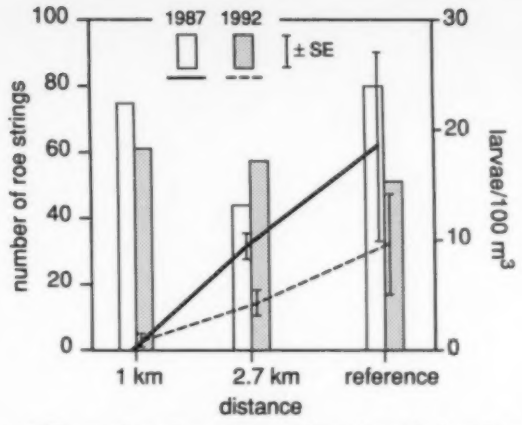

FIG. 4. The number of roe strings (columns) attached on the artificial spawning substrates and the number of perch larvae observed in Bongo net trawlings at exposed stations and at the reference site in 1987 and 1992.

1987 data (Karås et al. 1991). Evidently, perch frequently spawned close to the effluent discharge.

No dead eggs were observed when the roe samples were analysed in the laboratory. After separating the embryos from the egg, checks were made for external malformations. In exposed samples (stations 1 and 2) 2 and 1% of 200 embryos, respectively, were malformed compared with 0.5% of 200 in the reference area. The difference was not statistically significant. The 1987 study demonstrated a high prevalence of such injuries (10% of 200) close to the mill. The risk of embryo malformations thus appeared to be reduced.

When embryo lengths at stations 1 and 2 and at the reference site were compared in the 1987 study (Table 2), exposed fish were significantly shorter than the normal embryos. This difference still remained in 1992 (t-test, $p < 0.05$). When the 1987 and 1992 reference data were compared, there were no statistically significant differences, which verified that the size of a normal perch embryo was uniform over the years.

All weight estimates differed negatively in embryos from the effluent area when compared with the reference samples in 1987. In the more recent study, total weights from exposed stations 1 and 2 were not significantly different from those at the local reference site. Embryo weights without yolk sac, however, were significantly lower at the exposed sites (Table 2). Consequently, both length and weight of the embryos were still affected in the effluent area in 1992.

Larval Studies

In 1987, few perch larvae were caught in samplings close to the mill (Fig. 4), and all were in a late stage of development. Because the mill discharge is mixed with water pumped from a nearby bay (Fig. 1), these larvae may have been transported to the area after hatching. Further from the mill the abundance of larvae increased, although their numbers were still significantly lower than at the reference site (Mann–Whitney U-test, $p < 0.05$). In 1992, the distribution pattern corresponded with the earlier observations (Fig. 4) with significantly lower larval abundances close to the mill (Mann– Whitney U-test, $p < 0.05$). However, newly hatched yolk sac larvae were also seen close to the effluent outfall, indicating that some survival was now possible, and the difference in abundance between station 2 and the reference site was not significant. The study in 1992 was characterized by a very rapid temperature increase during the hatching period, and larvae occurred in all areas already at first sampling. The data therefore underestimate the initial number of larvae for the 1992 brood.

TABLE 2. Length (mm), total weight (mg), and weight without yolk sac (mg) in perch embryos from the Norrsundet effluent area compared to the local reference site. Data from 1987 from Karås et al. (1991).

	Length		Total weight		Weight without yolk sac	
Station	1987	1992	1987	1992	1987	1992
1	4.7	4.8	14.8	17.6	7.3	7.6
2		4.7		15.6		7.7
Reference	5.1	5.1	17.6	17.6	9.8	9.9

Larvae of species that were formerly rare near the mill were also identified, e.g., Baltic herring (*Clupea harengus*), different cyprinids (roach, *Rutilus rutilus*; bleak, *Alburnus alburnus*; ide *Leuciscus idus*; and rudd, *Scardinius erythrophtalmus*) and gobiids, *Pomatoschistus* spp. Catches of these species, however, were highest in the reference area.

Test Fishing

Catches from test fishing in 1982, 1985, 1987, and 1988 (Neuman and Karås 1988; Karås et al. 1991) were compared with those in 1992. During the first 2 yr, catches were low close to the mill and few species were observed (Table 3). Only three species (roach, perch and ruffe, *Gymnocephalus cernua*) occurred in the catches at station 1 in 1982 and 1985, and at station 2 only occasional catches were made of other species. In 1992, the number of species had increased in the catches. Thirteen species were noted at station 2, which was similar to the number of species at the reference sites. The abundance of fish, however, still appeared low when compared with the reference data (Table 3).

The distributions of the dominant species, perch and roach, in the effluent area in 1982 and 1985 were previously analysed (Neuman and Karås 1988). For perch, catch per unit effort was significantly lower at stations 1, 2, and 5 compared with the most distant station, which was considered to serve as a local reference site. Station 1 differed significantly from all other visited stations. When data from the later studies in 1987–1992 were compared, the catch pattern remained the same, although perch abundances seemed to have increased at stations 1 and 2 in 1992 (Fig. 5). If the effluent was no longer affecting perch abundance, there should have been no difference in catch per unit effort between station 1 and the reference. This hypothesis, however, was rejected because abundances were also significantly lower close to the mill outfall in 1992.

The second dominant species, roach, also occurred in comparably low numbers at station 1 (significantly lower than at all other sites in 1982; Neuman and Karås 1988). Further from the mill, there was a zone of high roach abundance all through the study period, interpreted as an effect of local eutrophication (Neuman and Karås 1988). In 1992,

TABLE 3. Catch per unit effort in gillnet fishing of the most common species at stations 1, 2, and 7 (NS 1, 2, and 7) at Norrsundet in 1982, 1985, 1987, 1988, and 1992, and in four reference areas (REF 1–4). Mean values are presented for the period 1989–1993 in reference area 1 and for 1983–1992 in reference area 4.

Area	Year	Roach	Perch	Ruff	Herring	Whitefish	Bream	Silverbream	Other	Total CPUE
NS 1	1982	<1	<1	<1	0	0	0	0	0	1
	1985	5	1	<1	0	0	0	0	0	6
	1987	2	1	0	0	<1	0	0	0	3
	1988	8	1	1	<1	0	<1	0	0	11
	1992	6	2	<1	<1	0	0	0	<1	9
NS 2	1982	4	1	3	0	0	0	0	0	8
	1985	33	2	1	1	0	<1	0	<1	36
	1987	13	4	3	<1	0	0	0	0	20
	1988	19	3	2	5	<1	<1	<1	<1	30
	1992	36	7	<1	8	0	<1	<1	1	53
NS 7	1992	21	27	1	2	0	0	1	<1	51
REF 1	1989–1993	76	38	3	3	1	0	0	<1	121
REF 2	1991	31	23	2	10	4	<1	0	<1	70
REF 3	1991	44	18	1	23	0	<1	12	<1	98
REF 4	1983–1992	50	20	3	27	0	0	0	<1	100

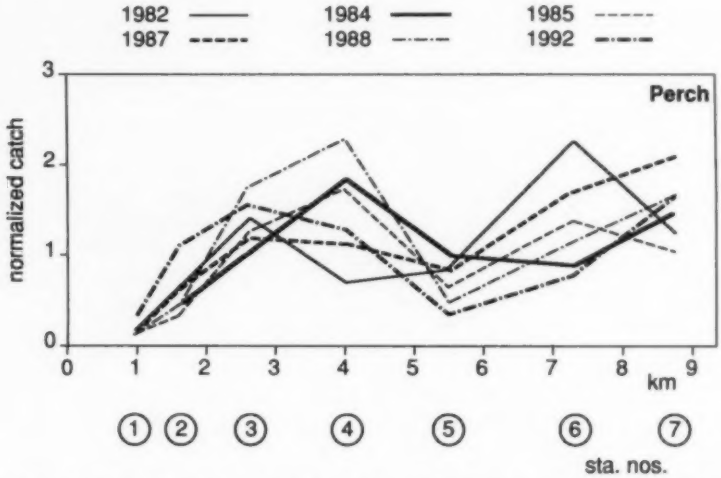

FIG. 5. Catch per unit effort of perch in test fishings made during the period 1982–1992. Circled nos. 1–7 on the *x*-axis are the sampling sites.

roach abundance had increased at station 1 and was not significantly different from the abundance at station 7.

Only a few malformed fish were observed in the visual examination of the net catches and their prevalence was not higher close to the mill. Fin erosion was not detected.

Discussion

Perch recruitment was strongly reduced during 1982–1985 when chlorine bleaching dominated at the Norrsundet mill (Neuman and Karås 1988) and the discharges of COD and suspended solids still were high. Although process improvements cut down the waste discharges, there were still pronounced effects causing high larval mortality close to the mill in 1987 (Karås et al. 1991). Until 1984, the main environmental action at the mill was to reduce COD and suspended solids in the effluent discharge below the limits set by regulations. After this was accomplished, only small changes occurred in these variables during the following years (Fig. 2). The regulatory activities shifted to the chlorinated waste, and AOX discharges were reduced from over 3 kg/t ADP to about 0.5 kg/t ADP in 1992 by stepwise process changes. Data covering a considerable period could thus be studied, during which the main change in effluent chemistry was related to bleaching improvements. Although the chlorine dioxide substitution was expected to reduce the effluent toxicity, the recovery of the Norrsundet perch population was not complete. The formerly obvious effects on sexual maturation and relative gonad size could no longer be seen, and embryo malformations were no longer significantly more abundant; but there was still high larval mortality accompanied by low embryo quality in the area within 1–2 kilometres of the effluent outfall, and the abundance of adult perch was low.

The effects on larval survival of perch indicate a continuing toxic impact. Whether this is related to current effluent toxicity or is an effect of persistent toxic material from effluent in the past cannot be judged. The capacity of this area to act as a trap for toxic materials is, however, considered to be low, as it is shallow and there are few places where sediments can accumulate (Södergren 1989). Moreover, the discharge of AOX was already low during 1988–1989. For these reasons, the results should reflect the more recent effluent situation, not the previous highly chlorinated effluents of the early 1980s. This suggestion is supported by a Norwegian study, demonstrating that the concentrations of organochlorines in fish had decreased to low levels a few years after mill shutdown (Carlberg et al. 1987).

As the studied population was sedentary (Sandström et al. 1988), some time may be required for possibly irreversibly damaged adult fish to die or be replaced by a healthy generation with full reproductive capacity after toxic exposure has stopped. The high mortality (over 50% per year; Sandström and Thoresson 1988) leading to rapid turnover of the adult stock and the possibility for immigration of less exposed fish from surrounding areas should, however, make this lag period short. Consequently, studies made in 1992 should mainly reflect recent effluent quality, suggesting that effluent from chlorine dioxide bleached kraft pulp production may reduce larval survival and harm fish recruitment.

A broad response pattern has been described in the area of the effluent since the research started in 1982. The observations included increased liver MFO activity, changes in blood cell patterns, decreased osmoregulatory capacity, fin erosion, stimulated growth, and high adult mortality (Andersson et al. 1988; Lindesjöö and Thulin 1990; Sandström et al. 1988; Sandström and Thoresson 1988), besides the effects on reproduction, adult abundance, and community structure discussed in this paper. Many environmental improvements have, however, been documented as the effluent chemistry was changed. During the first period of COD and suspended solids reductions, fin erosion ceased to appear, as was evident from the studies by Lindesjöö and Thulin (1990), which have been verified by the 1992 test fishings. In 1984, a severe pike (*Esox lucius*) jaw deformity was detected, but could no longer be seen in material collected in 1988 (Lindesjöö and Thulin 1992). Studies made after 1985 should reflect changes in the bleaching process, but in a follow-up study of biochemistry and physiology in 1988 and 1990, effects were still apparent when compared with the 1984–1985 data, though they were less pronounced (Förlin et al. 1991). Increased MFO activity was the most evident response.

Damage to reproductive capability may be difficult to detect by means of the commonly used GSI analysis. Significant effects on embryo size and larval abundance were still visible when relative gonad size estimates failed to demonstrate an impact. Damage to this population started with larval mortality, and in a sendentary species this can lead to reduction of the local stock, explaining the consistently low catch of adult perch.

This study did not contradict the hypothesis that molecular chlorine bleaching can harm the ecosystem, and the investments made to reduce organochlorine discharges seem to have been justified. However, significant ecological damage persists, supporting recent Canadian and Finnish observations that nonchlorinated components of kraft mill effluents may also be toxic (Lehtinen et al. 1990, 1991; Lindström-Seppä et al. 1992; Martel et al. 1993).

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References

Andersson, T., L. Förlin, J. Härdig, and Å. Larsson. 1988. Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents. Can. J. Fish. Aquat. Sci. 45: 1525–1536.

Carlberg, G.E., A. Kringstad, K. Martinsen, and O. Nashaug. 1987. Environmental impact of organochlorine compounds discharged from the pulp and paper industry. Pap. Puu 4: 337–341.

Förlin, L., T. Andersson, L. Balk, and Å. Larsson. 1991. Biochemical and physiological effects of pulp mill effluents on fish p. 235–243. *In* A. Södergren [ed.] Environmental Fate and Effects of Bleached Pulp Mill Effluents. Proceedings of a SEPA Conference, 19–21 Nov. 1991, Stockholm, Sweden. Rep. 4031. Swedish Environmental Protection Agency, Stockholm.

Hodson, P.V., D. Bussiéres, M.M. Gagnon, J.J. Dodson, C.M. Couillard, and J. Carey. 1991. Review of biochemical, physiological, pathological and population responses of white sucker (*Catostomus commersoni*) to BKME in the St. Maurice River, Quebec, p. 261–269. *In* A. Södergren [ed.] Environmental Fate and Effects of Bleached Pulp Mill Effluents. Proceedings of a SEPA Conference, 19–21 Nov. 1991, Stockholm, Sweden. Rep. 4031. Swedish Environmental Protection Agency, Stockholm.

Karås, P., E. Neuman, and O. Sandström. 1991. Effects of a pulp mill effluent on the population dynamics of perch, *Perca fluviatilis*. Can. J. Aquat. Fish. Sci. 48(1): 28–34.

Kruskal, W.H., and W.A. Wallis. 1952. Use of ranks in one-criterion analysis of variance. J. Am. Stat. Assoc. 47: 583–621.

Lehtinen, K.-J., A. Kierkegaard, E. Jakobsson, and A. Wandell. 1990. Physiological effects in fish exposed to effluents from mills with six different bleaching processes. Ecotoxicol. Environ. Saf. 19: 33–46.

Lehtinen, K.-J., A. Oikari, J. Hemming, K. Mattsson, and J. Tana. 1991. Effects on rainbow trout (*Oncorhyncus mykiss*) of food contaminated with solid fraction of bleached kraft mill effluents (BKME), p. 293–309. *In* A. Södergren [ed.] Environmental Fate and Effects of Bleached Pulp Mill Effluents. Proceedings of a SEPA Conference, 19–21 Nov. 1991, Stockholm, Sweden. Rep. 4031. Swedish Environmental Protection Agency, Stockholm.

Lindesjöö, E., and J. Thulin. 1990. Fin erosion of perch *Perca fluviatilis* and ruffe *Gymnocephalus cernua* in a pulp mill effluent area. Dis. Aquat. Org. 8: 119–126.

Lindesjöö, E., and J. Thulin. 1992. A skeletal deformity of northern pike, *Esox lucius* L., related to pulp mill effluents. Can. J. Fish. Aquat. Sci. 49: 166–172.

Lindström-Seppä, P., S. Huuskonen, M. Pesonen, P. Muona, and O. Hanninnen. 1992. Unbleached pulp mill effluents affect cytochrome P-450 monooxygenase enzyme activities. Mar. Environ. Res. 34: 157–161.

Martel, P.H., T.G. Kovacs, B.I. O'Connor, and R.H. Voss. 1993. A survey of pulp and paper mill effluents and their potential to induce mixed function oxidase enzyme activity in fish, p. A165–A178. *In* Proceedings, 79th Annual Meeting, January 1993. Technical Section, Canadian Pulp and Paper Association, Montreal, Que.

McMaster, E. 1991. Impact of bleached kraft mill effluent on fish populations in Jackfish Bay, Lake Superior. MSc thesis, University of Waterloo, Waterloo, Ont. 132 p.

Munkittrick, K.R., M.E. McMaster, C.B. Portt, G.J. Van Der Kraak, I.R. Smith, and D.G. Dixon. 1992. Changes in maturity, plasma sex steroid levels, hepatic mixed-function oxidase activity, and the presence of external lesions in lake whitefish (*Coregonus clupeaformis*) exposed to bleached kraft mill effluent. Can. J. Fish. Aquat. Sci. 49: 1560–1569.

Neuman, E., and P. Karås. 1988. Effects of pulp mill effluent on a Baltic coastal fish community. Water Sci. Technol. 20(2): 95–106.

Sandström, O., P. Karås, and E. Neuman. 1991a. Pulp mill effluent effects on species distributions and recruitment in Baltic coastal fish. Finn. Fish. Res. 12: 101–110.

Sandström, O., E. Neuman, and P. Karås. 1988. Effects of a bleached pulp

mill effluent on growth and gonad function in Baltic coastal fish. Water Sci. Technol. 20: 107–118.

SANDSTRÖM, O., E. NEUMAN, AND P. KARÅS. 1991b. Bleached pulp mill effects on Baltic coastal fish populations, p. 357–370. *In* A. Södergren [ed.] Environmental Fate and Effects of Bleached Pulp Mill Effluents. Proceedings of a SEPA Conference, 19–21 Nov. 1991, Stockholm, Sweden. Rep. 4031. Swedish Environmental Protection Agency, Stockholm.

SANDSTRÖM, O., AND G. THORESSON. 1988. Mortality in perch populations in a Baltic pulp mill effluent area. Mar. Pollut. Bull. 19(11): 564–567.

SÖDERGREN, A. [ed.]. 1989. Biological effects of bleached pulp mill effluents. Rep. 3558, National Swedish Environmental Protection Board, Stockholm.

THORESSON, G. 1993. Guidelines for coastal monitoring. Fishery biology. National Board of Fisheries, Institute of Coastal Research, Öregrund, Sweden. Kustrapport 1993: 1: 36 p.

ZAR, J.H. 1974. Biostatistical analysis. Prentice-Hall Inc., Englewood Cliffs, N.J.

Bioindicators of Stress in Winter Flounder, *Pleuronectes americanus*, Captured Adjacent to a Pulp and Paper Mill in St. George's Bay, Newfoundland

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Barker, D.E., R.A. Khan, and R. Hooper. 1994. Bioindicators of stress in winter flounder, *Pleuronectes americanus*, captured adjacent to a pulp and paper mill in St. George's Bay, Newfoundland. Can. J. Fish. Aquat. Sci. 51: 2203–2209.

Winter flounder, *Pleuronectes americanus*, inhabiting a marine inlet (Port Harmon) adjacent to a pulp and paper mill, and from a reference site (St. George's) 12 km away, were examined for signs of stress. Fish from Port Harmon showed significantly lower condition (*k*) factors, hepatosomatic indices, blood haemoglobin, and lymphocyte levels, than those from the reference site. Delayed spawning or spermiation were evident in female and male flounder, respectively, from Port Harmon compared with those from St. George's. Prevalence and intensity of larval anisakid nematodes, metacercariae of the digenetic trematode, *Cryptocotyle lingua*, and cysts of the microsporan, *Glugea stephani*, were significantly higher at Port Harmon than at St. George's, whereas prevalence and intensity of an intestinal acanthocephalan were significantly lower at Port Harmon. The percentage of flounder with external lesions (fin necrosis, ulcers) was significantly higher at Port Harmon than at St. George's. Anaemia, low lymphocyte levels, low somatic indices, high incidence of external lesions, and abnormal parasitic infestations are all suggestive of immunosuppression. Because basic water chemistry at the two sites was similar, the evidence of chronic stress observed in winter flounder from Port Harmon can most likely be attributed to pollutants from the effluent that have accumulated in the sediment.

Des plies rouges, *Pleuronectes americanus*, qui habitent dans un bras de mer (Port Harmon) contigu à une fabrique de pâte et papier, et d'autres prélevés dans une station témoin (St. George's) située à 12 km de distance, ont été examinées pour juger si elles présentaient des signes de stress. Chez les sujets de Port Harmon, on a constaté que les valeurs prises par les coefficients de condition (*k*), les indices hépatosomatiques, et la teneur en hémoglobine du sang ainsi que la numération lymphocytaire, étaient significativement inférieures à celles relevées chez les sujets de la station témoin. Le retard de la ponte ou de l'émission du sperme était évident chez les femelles et les mâles, respectivement, de Port Harmon, à comparer à la situation chez les sujets de St. George's. La fréquence et la densité des larves de nématodes anisakidés, des métacercaires du trématode digène, *Cryptocotyle lingua*, ainsi que des spores de la microsporidie, *Glugea stephani*, étaient significativement supérieures chez les sujets de Port Harmon à celui des sujets de St. George's, tandis que celles d'un acanthocéphale intestinal étaient significativement inférieures chez les sujets de Port Harmon. Le pourcentage de plies porteuses de lésions externes (nécrose des nageoires, ulcères) étaient significativement supérieur chez les sujets de Port Harmon à celui des sujets de St. George's. L'anémie, une numération lymphocytaire basse, les faibles valeurs des indices somatiques, le pourcentage élevé de lésions externes et des infestations anormales de parasites suggèrent tous l'immunosuppression. Puisque la chimie de l'eau est essentiellement la même aux deux stations, les signes d'un stress chronique chez la plie rouge de Port Harmon est probablement attribuable aux contaminants contenus dans les effluents qui se sont accumulés dans les sédiments.

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Several studies have reported the effects of pulp and paper effluent on fish captured downstream, in rivers, marine inlets, and coastal regions. These results have demonstrated weight loss, impaired liver function, skin lesions, fin necrosis, cellular hypertrophy, skeletal deformation, disruption of the immune system, presence of tumours, and changes in the parasite burden (Andersson et al. 1988; Bengtsson et al. 1988; Couillard et al. 1988; Khan et al. 1992; Lehtinen 1990; Lehtinen et al. 1984; Lindesjoo and Thulin 1990; Lindstrom-Seppa and Oikari 1990; Neuman and Karas 1988; Sandstrom and Thoresson 1988). Some of these studies also observed increased liver enzyme (mixed function oxygenase; MFO) activity in exposed fish captured near pulp mills (Andersson et al. 1988). Many toxic chemicals have been identified in effluents from pulp mill that use chlorine gas for bleaching, including chlorophenols, resin acids, guaiacols, furans, and dioxins (Bettis 1991; Waldichuck 1990).

Recent studies in Canada, especially in Lake Superior, have reported various responses in three species of fish exposed to bleached kraft pulp mill effluent. Lower levels of serum sex hormones and increased liver MFO levels were observed in white sucker (*Catostomus commersoni*), longnose sucker (*C. catostomus*), and lake whitefish (*Coregonus*

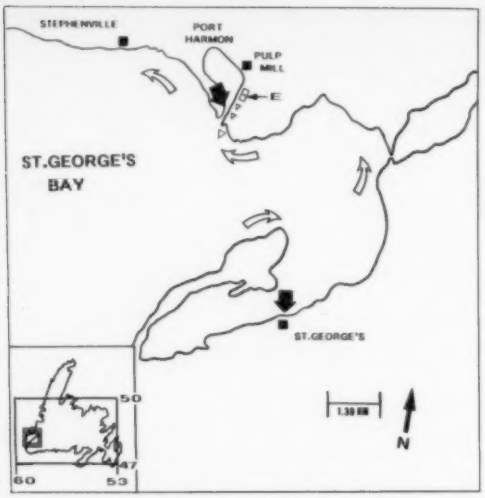

FIG. 1. Location (closed arrows) of the pulp mill (Port Harmon) and the reference site (St. George's) in St. George's Bay, Newfoundland. Open arrows show the direction of the ocean current. E = effluent holding pond.

clupeaformis) (McMaster et al. 1991; Munkittrick et al. 1991, 1992a, 1992b). Additionally, slower growth rates, smaller gonads, increased age to maturity, smaller egg size, and fewer secondary sexual characters in males were noted in white suckers near the discharge site than at a reference location upstream (McMaster et al. 1991). Another study, conducted at St. Maurice River, Quebec, revealed that white suckers living near to, and as far as 95 km downstream from, a bleached kraft mill showed elevated levels of MFO activity and enlarged livers, but the effects on reproduction were inconclusive (Hodson et al. 1992). While the results of these studies were comparable to those reported from Scandinavia (Andersson et al. 1988; Bengtsson et al. 1988; Lehtinen 1990; Lindstrom-Seppa and Oikari 1990; Neuman and Karas 1988; Sandstrom and Thoresson 1988), it appeared that the physiological changes observed in fish occurred after exposure to either chlorinated or sulphated pulp mill effluent.

In view of the above reports, the present study was undertaken to compare winter flounder (*Pleuronectes* = *Pseudopleuronectes americanus*) populations living adjacent to a pulp and paper mill in Newfoundland at Port Harmon with those from a reference site (St. George's) using several bioindicators of stress (Adams 1990). Techniques used in the present study include condition factor, organ somatic indices, blood values (haemoglobin and lymphocyte numbers), and levels of parasitic infestation.

Materials and Methods

Site Description And Winter Flounder Biology

Samples were collected from Port Harmon (48°31′N, 58°33′W) (−1 km from the effluent discharge site) and near the town of St. George's (48°26′N, 58°30′W) approximately 12 km from the effluent site (Fig. 1). Based on the direction of currents in St. George's Bay, the sample site at St. George's should be effluent free (water current data from Canadian Coast Guard). The bottom (3–10 m) substrate at Port Harmon consisted mainly of soft, muddy sediment occasionally mixed with wood fibres. In contrast, the bottom (2–6.5 m) substrate at the St. George's sample site consisted of fine sand interspersed with occasional rock projections. The faunal (bivalves, annelids, arthropods, and five fish species, viz., winter flounder, *Myoxocephalus scorpius*, *M. octodecimspinosus*, *Tautogolabrus adspersus*, and *Microgadus tomcod*) and floral (*Laminaria*, *Fucus*, *Chorda*, *Desmarestia*, *Chundrus*, *Ceramium*, *Ulva*, and *Zostea* spp.) representatives were common to both sample sites, but densities or species abundances were not quantified. Both sites were areas of fish offal disposal, providing ample foraging opportunities for the local fish assemblages.

The mill at Port Harmon first operated from 1974 to 1977 as a linerboard mill, during which time chlorine was used for bleaching the pulp. It reopened in 1981 for newsprint production that uses thermomechanical (TMP) pulp mixed with a maximum of 10% prebleached imported kraft pulp (Abitibi-Price 1981*a*, 1981*b*). Stock pulp is bleached currently with sodium hydrosulphite. The effluent is first temporarily stored in a settling pond, where it receives primary treatment (aeration), then is discharged (pipe size: 1.5 m diameter, located 5 m deep) at an average rate of 1.8×10^4 m^3/d, directly into St. George's Bay, adjacent to the marine inlet, Port Harmon (Fig. 1). Analysis of the effluent and sediment from the collecting site at Port Harmon in June 1992 revealed low levels (<10 mg/L) of resin acids (i.e., dihydroabietic acid) but data from 1990 suggest that there is considerable fluctuation (as high as 1×10^3 mg/L) in the discharged effluent (Environment Canada, unpublished data). No detectable levels of resin acids were observed in samples from St. George's. A comparison of water characteristics including temperature, salinity, conductivity, pH, and oxygen at the surface and bottom (6 m) taken during the months of May, June, July, August, and November 1992 from Port Harmon and St. George's revealed no major differences (Barker 1993).

The marine inlet of Port Harmon supports a year-round population of winter flounder, a commercially important flatfish and common inhabitant of inshore areas. In Newfoundland waters, adult winter flounder migrate to shallow water (<15 m) during autumn, cease feeding, and burrow into the substrate where they will remain until emerging to spawn in spring (Van Guelpen and Davis 1979). Winter flounder prefer temperatures less than 14°C in north temperate waters, and large adults (>26 cm) will often migrate to deeper (>6 m), cooler, inshore waters during summer (Scott and Scott 1988). No information regarding the migration of winter flounder in the St. George's Bay region has been published; however, preliminary results of a tagging study in this area have been unable to demonstrate the existence of any reciprocal migration between flounder at Port Harmon and St. George's (D.E. Barker and R.A. Khan, unpublished data).

Winter flounder use visual cues for foraging and feeding on a variety of benthic prey including gastropods, bivalves, polychaetes, crustaceans, and fish (Keats 1990; MacDonald and Waiwood 1987). MacDonald and Waiwood (1987) calculated a daily feeding ration for winter flounder as 1.77% body weight/d during the summer, when feeding rates are highest. Because a wide variety of common prey is consumed, flounder tend to be both definitive and intermediate hosts of several protozoan and helminth parasites (Margolis and Arthur 1979; Ronald 1963; Scott 1982, 1985; Takvorian and Cali 1984).

TABLE 1. Mean (±SE) length, weight, *k*-factor, organ somatic indices, and blood values of *P. americanus* collected from Port Harmon (effluent site) and St. George's (reference site) during 1991 and 1992.

Variable (Year)	Port Harmon	(*n*)	St. George's	(*n*)
Length (1991 Summer)+	23.2±0.9	(40)	22.8±1.2	(18)
(cm) (1992 Spring)	29.7±0.6	(114)	26.6±0.5	(99)**
(1992 Summer)	25.3±0.5	(103)	22.1±0.7	(26)**
Weight (1991 Summer)	149.5±24.2	(40)	156.9±9.0	(18)
(g) (1992 Spring)	274.3±15.3	(114)	194.8±12.3	(99)***
(1992 Summer)	191.2±13.4	(103)	131.8±12.8	(26)***
k-factor (1991-Summer)	0.97±0.03	(40)	1.16±0.02	(18)***
(1992 Spring)	0.92±0.01	(114)	0.93±0.01	(99)
(1992 Summer)	1.01±0.01	(103)	1.17±0.02	(26)***
HSI++ (1992 Spring)	1.24±0.04	(108)	0.99±0.04	(96)***
(1992 Summer)	1.41±0.05	(67)	1.65±0.11	(26)**
GSI (1992 Spring ♀)	18.87±0.81	(33)	15.16±0.74	(19)**
(1992 Spring ♂)	7.48±0.57	(24)	5.61±0.25	(24)**
VSI (1992 Spring)	7.70±0.37	(108)	6.95±0.30	(96)
(1992) Summer)	7.48±0.35	(67)	9.06±0.48	(26)***
Hb (1992 Spring)	4.17±0.17	(62)	4.75±0.19	(55)**
(1992 Summer)	3.71±0.21	(59)	4.83±0.29	(26)***
Lymphocyte (1992 Spring)	13.77±1.98	(62)	50.63±9.78	(55)***
(/1000 cells) (1992 Summer)	42.81±2.03	(20)	79.62±5.74	(26)***

+Summer = June–September; Spring = May. ++HSI = hepatosomatic index; GSI = gonadosomatic index; VSI = visceralsomatic (= gastrointestinal) index; Hb = hemoglobin (g/100 mL).

***, $p < 0.001$; **, $0.01 < p < 0.05$.

Methods

Monthly samples of winter flounder were collected from spring to autumn of 1991 and 1992 from both Port Harmon and St. George's. No samples were obtained during winter, as both sample sites were covered with ice. All flounder were collected from depths of 2–10 m, either by SCUBA divers using a dipnet (8-cm mesh size), or by hook and line. During the fish collection dives of 1992, water samples were collected in plastic 125-mL bottles and temperatures were recorded by SCUBA divers at depths of 0, 3, 6, and 9 m. Each water sample was analyzed for salinity, conductivity, dissolved oxygen, and pH, using both a portable YSI meter and a Hach™ kit.

Sampled fish were killed via spinal severance and blood was immediately taken using a 3-mL heparinized syringe and needle (Khan et al. 1992). A thin blood smear was made from each blood sample and haemoglobin levels were determined by a haemoglobinometer (American Optical™). Blood smears were fixed with methanol and stained with Giemsa. The total length of each fish was recorded as well as the dressed (eviscerated) weight, and organ weights which included liver, spleen, gonad, and gastrointestinal tract. The latter was examined for parasites, which were enumerated and identified to the lowest possible taxon (Moller and Anders 1986; Schmidt 1988). In addition, the viscera of each fish were examined macroscopically for the presence of cysts from a parasitic microsporan (*Glugea stephani*) (Takvorian and Cali 1984). The encysted metacercariae of *Cryptocotyle lingua* were enumerated from the following external regions of each fish: (1) right pectoral fin, (2) right pelvis fin, (3) 1st 10 posterior fin rays of the dorsal and anal fins, and (4) 1st five dorsal fin rays of the caudal fin. Necrosis of the caudal fin was expressed as a percentage of the caudal fin eroded per total length of fin. Any other areas of fin necrosis or external lesions (i.e., ulcers) on each fish were also noted.

Data were analyzed using two computer software packages, SPSS™ and MINITAB™. Values such as the condition (*k*) factor ($W/L^3 \times 100$, using dressed weight), organ somatic index (organ weight/dressed weight × 100), and percentage of fin eroded (necrotic length/caudal length × 100) were compared between the two groups of fish using a one-way ANOVA, with a Duncan's multiple-groups test (Sokal and Rohlf 1987). Prevalence of parasites was compared between the groups using a *G*-test as outlined in Sokal and Rohlf (1987). Finally, a simple linear regression was used to test for correlation between length and weight (Sokal and Rohlf 1987). For each of the above tests, differences were considered significant when $p < 0.05$.

Results

Four hundred winter flounder, including both juvenile and adult fish, were collected during 1991–1992, of which 257 originated from Port Harmon, and 143 from St. George's. A simple linear regression of weight (dressed) and caudal length of flounder from each site failed to demonstrate any statistical difference between both equations. In addition, there were no differences in length–weight regressions between males and females, or in sex ratios, as tested by a *G*-test. The 1992 pooled sample of flounder from Port Harmon was significantly greater in length than the 1992 pooled sample of flounder from St. George's.

Comparison of Body Condition and Organ Indices

A comparison of body condition and organ somatic indices revealed significant differences between groups of winter flounder collected from Port Harmon and St. George's. Mean

TABLE 2. Fin necrosis frequency (%) and mean (± SE) number of necrotic sites on *P. americanus* collected from Port Harmon (effluent site) and St. George's (reference site).

Variable	Port Harmon	St. George's
Caudal fin necrosis frequency (%) (1991%)	53 (57)	35** (11)***
Frequency of fish with >1 necrotic site (%)	20	6***
Mean no. of necrotic sites ($\bar{x}$ ± SE)	0.78±0.06	0.41±0.06***
% of caudal fin eroded ($\bar{x}$ ± SE)	15.9±1.4	9.9±1.7**
Dorsal and anal fin necrosis (%)	30	19**
Prevalence of skin ulcers (%)	11	5**
n	201	108

***$p < 0.001$, **$0.001 < p < 0.05$.

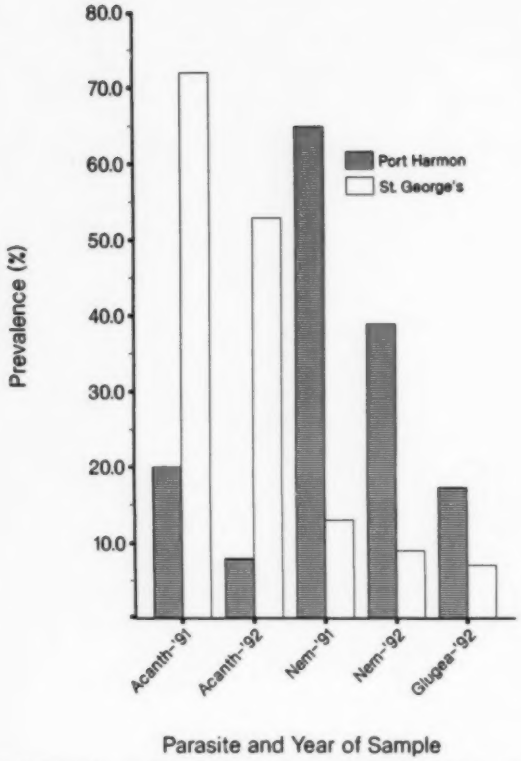

FIG. 2. Prevalence (%) of intestinal Acanthocephala, Nematoda, and Microspora (*Glugea*) in *P. americanus* from Port Harmon (1991, $n = 40$; 1992, $n = 159$) and St. George's (1991, $n = 18$; 1992, $n = 105$).

condition factor, hepatic index, and gastrointestinal index were significantly greater in fish taken at St. George's than from Port Harmon during summer, while during spring, mean hepatic indices were significantly greater in fish taken at Port Harmon (Table 1). However, gonadal (male and female) indices were significantly greater in flounder originating from Port Harmon than in those from St. George's. Examination of stage of gonadal maturity during May–June revealed that there were more mature fish taken at Port Harmon, whereas more postspawned–spermiated flounder occurred at St. George's. Additionally, during July and August, 12% of the flounder, both males and females, at Port Harmon had fully developed gonads, while all of the flounder from St. George's were spent. No difference was observed in the mean oocyte diameter or the mean number of oocytes from females of both sites. Additionally, no difference was observed in the splenosomatic indices of both groups of flounder.

Blood Values

A comparison of blood values revealed differences between winter flounder taken from the two sites. Haemoglobin and lymphocyte levels were significantly greater in fish taken at St. George's than those from Port Harmon (Table 1).

Prevalence of Fin Necrosis

Fin necrosis was observed more often in winter flounder taken at Port Harmon than at the St. George's site (Table 2). While the percentage of fish with this condition increased from May to August at both sites, it was significantly greater at Port Harmon (53%) than at St. George's (35%). Similarly, the percentage of fish with dorsal and anal fin necrosis was significantly greater at the discharge (30%) than at the reference (19%) site. Moreover, the prevalence and mean number of multiple necrotic sites per flounder were greater (16%, $\bar{x} = 0.8 \pm 0.06$) at Port Harmon than at St. George's (10%, $\bar{x} = 0.4 \pm 0.06$). Additionally, skin ulcers, which occurred primarily on the left side, were more common on flounder from Port Harmon (11%) than those from St. George's (5%).

Feeding and Parasite Burden

Flounder at both sites fed on similar types of prey, viz., amphipods, bivalves, fish offal, fish eggs, gastropods, polychaetes, isopods, decapods. However, the frequency with which several prey were ingested varied between the two sample sites. Flounder at Port Harmon fed primarily on fish offal, whereas at St. George's, they fed most frequently on herring (*Clupea harengus*) eggs and amphipods (Barker 1993).

Similar parasites were found in flounder collected from both sites but several differences in prevalence and intensity were observed. The prevalence and intensity of the gastrointestinal acanthocephalan *Echinorhynchus gadi* were significantly greater in samples from St. George's than from Port Harmon (Figs. 2 and 3). However, the prevalence and intensity of larval anisakid nematodes (found in the mesentery and intestine), the ectoparasitic metacercariae of *C. lingua*, and visceral cysts of the microsporan *G. stephani* were all significantly greater in flounder taken at Port Harmon than at St. George's (Figs. 2–4). Additionally, the mean intensity of metacercariae of *C. lingua* on both pectoral and caudal fins and on the gill filaments was greater in flounder

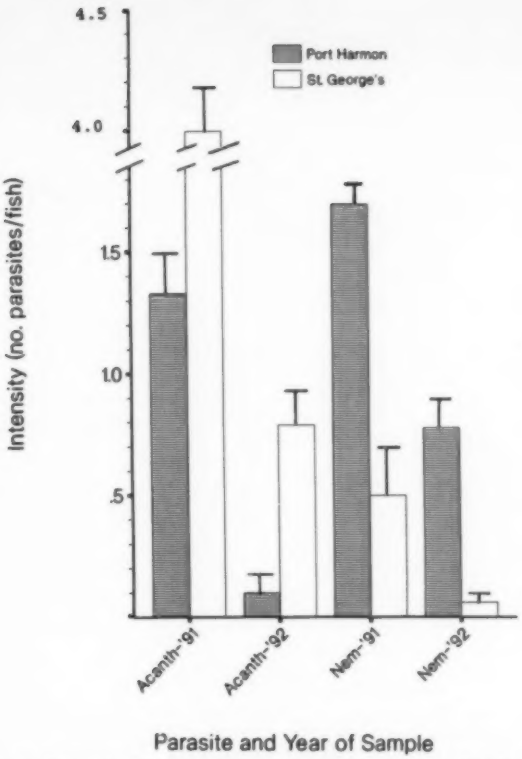

FIG. 3. Mean (± SE) intensity (total number of parasites/total number of fish examined) of intestinal Acanthocephala and Nematoda in *P. americanus* from Port Harmon (1991, n = 40; 1992, n = 159) and St. George's (1991, n = 18; 1992, n = 105).

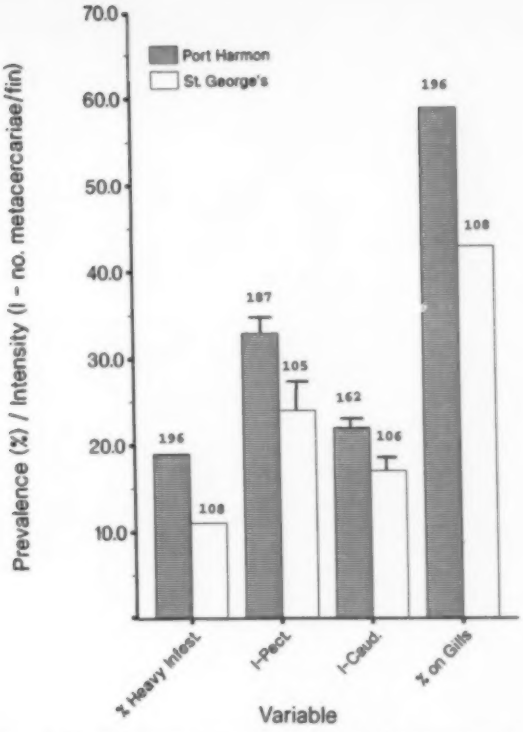

FIG. 4. Prevalence and mean intensity of *Cryptocotyle lingua* infestation on the pectoral and caudal fins and on the gill filaments of *P. americanus*.

captured at Port Harmon than those from St. George's (Fig. 4). Cysts (xenomas) of *G. stephani* were located on the external wall of the gastrointestinal tract of flounder taken at St. George's and varied in diameter from 2 to 12 mm, whereas at Port Harmon, in addition to this organ, cysts were also observed in the liver, spleen, kidney, and testis, and varied in diameter from 2 to 28 mm. No difference was observed in the prevalence and intensity of gastrointestinal digenetic trematodes from flounder originating from both sites.

Discussion

Results from the present study provide evidence of physiological changes in winter flounder living at Port Harmon, a site where effluent from a pulp and paper mill is discharged. Some of these alterations (including depressed condition factor, organ somatic indices, and blood values; increased prevalence of fin necrosis; and some parasitic infestations) are all indicative of impaired health and confirm previous findings (Khan et al. 1992). Low condition factor and hepatosomatic index only in summer might be attributed to a dietary deficiency and (or) the inhibitory effect of the effluent on feeding and (or) digestion. In contrast to these findings, McMaster et al. (1991) observed higher condition factors and liver somatic indices in white suckers (*Catostomus commersoni*) exposed to bleached kraft mill effluent (BKME) than in reference fish, and suggested a connection between disruptive metabolism and exposure to BKME. An increase in liver somatic indices is associated with elevated levels of MFO (McMaster et al. 1991). Several studies have shown elevated hepatic MFO activity or cytochrome P-450 after exposure to bleached and unbleached pulp and paper mill effluent (Andersson et al. 1988); Lindstrom-Seppa et al. 1992; McMaster et al. 1991; Munkittrick et al. 1991, 1992b). However, Lehtinen (1990) hypothesized that unbleached mill effluent had an inhibitory effect whereas BKME had a stimulatory effect on detoxifying enzyme systems. We attribute the lower HSI values during summer in flounder in the present study to depletion of stored energy (lipid and glycogen) based on histochemical findings (Khan et al. 1994), whereas in spring the higher values might be related to MFO activity. Bucher et al. (1992) also reported depletion of energy reserves in liver cells of sculpins (*Cottus gobio*) exposed to different levels of pulp mill effluent.

A higher percentage of postspawned females and significantly lower gonadosomatic indices in winter flounder from St. George's indicates delayed spawning in fish from Port Harmon. Andersson et al. (1988) reported reduced gonadal growth and impaired gonadal function in perch (*Perca fluviatilis*) inhabiting coastal waters in Sweden polluted by BKME. Studies on white suckers and lake whitefish exposed to BKME revealed reduced gonadal size, delayed age to maturity, and low levels of plasma sex steroids (McMaster et al. 1991; Munkittrick et al. 1992a). Secondary treatment of BKME and mill shutdown were successful in reducing the

numbers of fish with low plasma steroid levels (Munkittrick et al. 1992a).

Anaemia and low lymphocyte levels, noted in flounder at Port Harmon, are indicative of impaired health. Khan et al. (1992) also observed low haemoglobin values and a high prevalence of fin necrosis in flounder taken at the same site. The high prevalence of fin necrosis might have contributed to blood loss and, ultimately, anaemia. Mahoney and McNulty (1992) noted low haemoglobin and haematocrit levels in winter flounder with fin rot disease taken from an industrially polluted site. Andersson et al. (1988) also reported low levels of leucocytes in perch inhabiting waters near a bleached kraft mill and suggested that the immune systems of the fish were impaired. The presence of cysts of the microsporan *G. stephani* in several visceral organs of flounder taken at Port Harmon, in contrast to their restriction to the wall of the digestive tract in samples from St. George's, as noted in other studies (Evans and Gratzek 1989; Takvorian and Cali 1984), is suggestive of immunosuppression following chronic exposure to the pulpmill effluent. It is also possible that the high prevalence and intensity of larval nematodes and ectoparasitic digenean metacercariae in flounder taken near the pulp mill is associated with increased susceptibility to infection. Previous studies have revealed that many parasitic infestations increase in prevalence and intensity in fish exposed to sublethal levels of xenobiotics (Khan and Thulin 1991).

Acanthocephalan (*E. gadi*) infestations, unlike microsporan infections, were higher in flounder from St. George's and were often absent in samples of flounder from Port Harmon. A previous study by Khan et al. (1992) reported an absence of acanthocephalans in flounder collected from Port Harmon in 1990, compared with 41% prevalence in 1978, shortly after closure of the linerboard mill. Our data also indicate that the seasonal prevalence of acanthocephalan infestation in winter flounder sampled at St. George's was similar to that reported by Ronald (1963). The differences in prevalence and intensity noted between polluted and reference sites, could be associated with physiological changes in the intermediate and (or) definitive hosts. These include differential consumption of amphipods (*Gammarus* spp.), which can act as intermediate hosts (Moller and Anders 1986) and (or) interruption of the life cycle in the latter by toxicity of the effluent to larval stages. The former appears unlikely, as amphipods were consumed by groups of flounder from both sites. Examination of amphipods at Port Harmon and St. George's might reveal whether or not acanthocephalan larval stages develop at both sites. Because marine fish drink seawater to osmoregulate, it is possible that at Port Harmon acanthocephalans were voided from the gastrointestinal tract after direct contact with the ingested effluent and (or) associated changes in their host's physiology. These findings provide evidence to support the view that acanthocephalans can be used as bioindicators of pollution (Khan and Thulin 1991). Khan and Kiceniuk (1983) also reported a decrease in acanthocephalan (*E. gadi*) infestation of Atlantic cod (*Gadus morhua*) exposed to sublethal concentrations of petroleum hydrocarbons. However, Valtonen and Koskivaara (1989) reported an increase in acanthocephalan infestations in roach (*Rutilus rutilus*) living in a freshwater lake exposed to BKME.

These changes that occurred only in winter flounder captured in the vicinity of the outfall of a pulp and paper mill are all indicative of chronic stress. Moreover, it is likely that these effects are associated with xenobiotics that have accumulated in the sediment in which the fish were frequently submerged rather than in the water column. Additionally, evidence from a histopathological study suggests that these stress-related alterations increased with the age of the fish and were less pronounced in pelagic fish species (Khan et al. 1994). Future studies should focus on chemical analysis of the sediment and tissues of affected fish in order to establish a cause and effect relationship between specific pollutants in the effluent and stress-related symptoms in flounder.

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References

ADAMS, S.M. 1990. Status and use of biological indicators for evaluating the effects of stress on fish. *In* S.M. Adams [ed.] Biological indicators of stress in fish. Am. Fish. Soc. Symp. 8: 1–8.

ANDERSSON, T., L. FORLIN, J. HARDIG, AND A. LARSSON. 1988. Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents. Can. J. Fish. Aquat. Sci. 45: 1525–1536.

ABITIBI-PRICE. 1981a. Abitibi-Price: Stephenville newsprint. Pamphlet. Abitibi-Price Fine Papers, Stephenville, Nfld. 2 p.

ABITIBI-PRICE. 1981b. Abitibi-Price: Stephenville newsprint mill tour guide. Pamphlet. Abitibi-Price Fine Papers, Stephenville, Nfld. 2 p.

BARKER, D.E. 1993. Evidence of chronic stress in winter flounder, *Pleuronectes* (=*Pseudopleuronectes*) *americanus* living adjacent to a pulp and paper mill in St. George's Bay, western Newfoundland. M.Sc. thesis. Memorial University of Newfoundland, St. John's, Nfld. 101 p.

BENGTSSON, B.E., A. BENGTSSON, AND V. TJARNLUND. 1988. Effects of pulp mill effluents on vertebrae of fournhorn sculpin, *Myoxocephalus quadricornis*, bleak, *Alburnus alburnus*, and perch, *Perca fluviatilis*. Arch. Environ. Contam. Toxicol. 17: 789–797.

BETTIS, J. 1991. Bleach plant modifications, controls help industry limit dioxin formation. Pulp Pap. 65: 76–82.

BUCHER, F., R. HOFFER, AND W. SALVENMOSER. 1992. Effects of treated paper mill effluents on hepatic morphology in male bullhead (*Cottus gobio* L.). Arch. Environ. Contam. Toxicol. 23: 410–419.

COUILLARD, C.M., R.A. BERMAN, AND J.C. PANISSET. 1988. Histopathology of rainbow trout exposed to a bleached kraft pulp mill effluent. Arch. Environ. Contam. Toxicol. 17: 319–323.

EVANS, D.L., AND J.B. GRATZEK. 1989. Immune defense mechanisms in fish to protozoan and helminth infections. Am. Zool. 29: 409–418.

HODSON, P.V., M. MCWHIRTER, K. RALPH, B. GRAY, D. THIVERGE, J.H. CAREY, G. VAN DER KRAAK, M. WHITTLE, AND M.C. LEVESQUE. 1992. Effects of bleached kraft mill effluent on fish in the St. Maurice River, Quebec. Environ. Toxicol. Chem. 11: 1635–1651.

KEATS, D.W. 1990. Food of winter flounder, *Pseudopleuronectes americanus*, in a sea urchin dominated community in eastern Newfoundland. Mar. Ecol. Prog. Ser. 60: 13–22.

KHAN, R.A., D. BARKER, R. HOOPER, AND E.M. LEE. 1992. Effect of pulp and paper effluent on a marine fish, *Pseudopleuronectes americanus*. Bull. Environ. Contam. Toxicol. 48: 449–456.

KHAN, R.A., D.E. BARKER, K. RYAN, E.M. LEE, AND K. NAG. 1994. Histopathology in winter flounder (*Pleuronectes americanus*) living adjacent to a pulp and paper mill. Arch. Environ. Contam. Toxicol. 26: 95–102.

KHAN, R.A., AND J. KICENIUK. 1983. Effects of crude oil on the gastrointestinal parasites of two species of marine fish. J. Wildl. Dis. 19: 253–258.

KHAN, R.A., AND J. THULIN. 1991. Influence of pollution on parasites of aquatic animals. Adv. Parasitol. 30: 201–238.

LEHTINEN, K.-J. 1990. Mixed-function oxygenase enzyme responses and

physiological disorders in fish exposed to kraft pulp mill effluents: a hypothetical model. Ambio 19: 259–265.

LEHTINEN, K.-J., M. NOTINI, AND L. LANDLER. 1984. Tissue damage and parasite frequency in flounders, *Platichthys flesus*, chronically exposed to bleached kraft pulp mill effluents. Ann. Zool. Fenn. 21: 23–38.

LINDESJOO, E., AND J. THULIN. 1990. Fin erosion of perch, *Perca fluviatilis*, and ruffe, *Gymnocephalus cernua*, in a pulp mill effluent area. Dis. Aquat. Org. 8: 119–126.

LINDSTROM-SEPPA, P., S. HUUSKONEN, M. PESONEN, P. MUONA, AND O. HANNINEN. 1992. Unbleached pulp mill effluents affect cytochrome P-450 monooxygenase enzyme activities. Mar. Environ. Res. 34: 157–161.

LINDSTROM-SEPPA, P., AND A. OIKARI. 1990. Biotransformation and other toxicological and physiological responses in rainbow trout (*Salmo gairdneri*) caged in a lake receiving effluents of pulp and paper industry. Aquat. Toxicol. 16: 187–204.

MACDONALD, J.S., AND K.G. WAIWOOD. 1987. Feeding chronology and daily ration calculations for winter flounder (*Pseudopleuronectes americanus*), American plaice (*Hippoglossoides platessoides*), and ocean pout (*Macrozoarces americanus*) in Passamaquoddy Bay, New Brunswick. Can. J. Zool. 65: 499–503.

MAHONEY, J.B., AND J.K. MCNULTY. 1992. Disease-associated blood changes in normal seasonal hematological variation in winter flounder in the Hudson–Raritan estuary. Trans. Am. Fish. Soc. 121: 261–268.

MARGOLIS, L., AND J.R. ARTHUR. 1979. Synopsis of the parasites of fishes of Canada. Bull. Fish. Res. Board Can. 199: 269 p.

MCMASTER, M.E., G.J. VAN DER KRAAK, C.B. PORTT, K.R. MUNKITTRICK, P.K. SIBLEY, AND D.G. DIXON. 1991. Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels, and age at maturity of a winter sucker (*Catostomus commersoni*) population exposed to a bleached kraft pulp mill effluent. Aquat. Toxicol. 21: 199–218.

MOLLER, H., AND K. ANDERS. 1986. Diseases and parasites of marine fishes. Kiehl, Copenhagen. 365 p.

MUNKITTRICK, K.R., G.J. VAN DER KRAAK, M.E. MCMASTER, AND C.B. PORTT. 1992a. Relative benefit of secondary treatment and mill shutdown on mitigating impacts of bleached kraft mill effluent (BKME) on MFO activity and serum steroids in fish. Environ. Toxicol. Chem. 11: 1427–1439.

MUNKITTRICK, K.R., M.E. MCMASTER, C.B. PORTT, G.J. VAN DER KRAAK, I.R. SMITH, AND D.G. DIXON. 1992b Changes in maturity, plasma sex steroid levels, hepatic mixed-function oxygenase activity, and the presence of external lesions in lake whitefish (*Coregonus clupeaformis*) exposed to bleached kraft mill effluent. Can. J. Fish. Aquat. Sci. 49: 1560–1569.

MUNKITTRICK, K.R., C.B. PORTT, G.J. VAN DER KRAAK, I.R. SMITH, AND D.A. ROKOSH. 1991. Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. Can. J. Fish. Aquat. Sci. 48: 1371–1380.

NEUMAN, E., AND P. KARAS. 1988. Effects of pulp mill effluent on a Baltic coastal fish community. Water Sci. Technol. 20: 95–106.

RONALD, K. 1963. The metazoan parasites of the heterosomata of the Gulf of St. Lawrence. VII. Nematoda and Acanthocephala. Can. J. Zool. 41: 15–20.

SANDSTROM, O., AND G. THORESSON. 1988. Mortality in perch populations in a Baltic pulp mill effluent area. Mar. Pollut. Bull. 19: 564–567.

SCHMIDT, G.D. 1988. Essentials of parasitology. William C. Brown Publishers Inc., Dubuque, Iowa. 294 p.

SCOTT, J.S. 1982. Digenean parasite communities in flatfishes of the Scotian Shelf and southern Gulf of St. Lawrence. Can. J. Zool. 60: 2804–2811.

SCOTT, J.S. 1983. Digenean (Trematoda) populations in winter flounder (*Pseudopleuronectes americanus*) from Passsamaquoddy Bay, New Brunswick, Canada. Can. J. Zool. 63: 1699–1705.

SCOTT, W.B., AND M.G. SCOTT. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. 219: 731 p.

SOKAL, R.R., AND F.J. ROHLF. 1987. Introduction to Biostatistics. 2nd ed. W.H. Freeman & Co., New York, N.Y. 363 p.

TAKVORIAN, P.M., AND A. CALI. 1984. Seasonal prevalence of the microsporidan, *Glugea stephani* (Hagenmuller), in winter flounder, *Pseudopleuronectes americanus* (Walbaum), from the New York – New Jersey Lower Bay Complex. J. Fish Biol. 24: 655–663.

VALTONEN, E.T., AND M. KOSKIVAARA. 1989. Effects of effluent from a pulp and paper mill on parasites of the roach in central Finland, p. 163–168. *In* Soviet–Finnish Symposium on Fish Parasites of Northwestern Europe, 10–14 Jan. 1989. Petrozavodsk, USSR.

VAN GUELPEN, L., AND C.C. DAVIS. 1979. Seasonal movements of the winter flounder, *Pseudopleuronectes americanus*, in two contrasting inshore locations in Newfoundland. Trans. Am. Fish. Soc. 108: 26–37.

WALDICHUCK, M. 1990. Dioxin pollution near pulp mills. Mar. Pollut. Bull. 21: 365–366.

Detecting Small-scale Spatial Patterns in Lotic Predator–Prey Relationships: Statistical Methods and a Case Study

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We mapped the exact locations of two predaceous stream insects, a caddis larva (*Rhyacophila obliterata*) and a stonefly nymph (*Isoperla grammatica*), in 1-m^2 sampling windows in relation to their major prey, larval black flies (mostly *Simulium sublacustre*), which were quantified within the same areas by extensive systematic sampling. In mid-June, when black flies were abundant and highly clustered, *R. obliterata* was also aggregated on a scale of 5–10 cm. After the emergence of black flies in the beginning of July, the point pattern of the rhyacophilid was random. *Isoperla grammatica* was abundant only on the earlier sampling occasion, at which time it was randomly distributed. We also analysed spatial association between the two predators and found a weak indication of small-scale inhibition between them. In the range of current speeds of 40–60 cm/s, microhabitat overlap between the caddisfly and black fly was intensive, leading to high consumption of this semi-sessile prey. The second-order methods used proved powerful in detecting small-scale spatial patterns and could be more widely used by ecologists in place of classical single-statistic indices whenever detailed mapping of individuals is feasible.

Nous avons cartographié avec précision les emplacements où sont trouvés deux insectes prédateurs qui vivent dans des cours d'eau, une larve de la phrygane (*Rhyacophila obliterata*) et une nymphe de la perle (*Isoperla grammatica*), dans des parcelles d'échantillonnage de 1 m^2, en fonction de leur proie principale, les larves de la simulie, dont on a mesuré l'abondance dans les mêmes secteurs en pratiquant un échantillonnage systématique extensif. À la mi-juin, lorsque les simulies sont abondantes et fortement regroupées, *R. obliterata* s'agrège aussi à l'échelle de 5 à 10 cm. Après l'émergence des simulies au commencement de juillet, la distribution ponctuelle des rhyacophilidés est aléatoire. *Isoperla grammatica* n'était abondante que lors de la première période d'échantillonnage; à cette époque, elle était distribuée au hasard. Nous avons aussi analysé les rapports dans l'espace entre les deux prédateurs, et nous avons trouvé une mince indication d'une inhibition à petite échelle entre les deux. Avec des courants dont la vitesse se situe entre 40 et 60 $cm \cdot s^{-1}$, il y a une importante superposition des microhabitats de la phrygane et de la simulie, ce qui conduit à une forte consommation de cette proie semi-sessile. Les méthodes du second ordre qui ont été appliquées se sont révélées très efficaces dans la détection de la répartition dans l'espace des insectes à petite échelle; les écologistes pourraient y faire davantage appel au lieu d'appliquer la méthode classique par indices à une seule statistique, chaque fois qu'il est possible de cartographier en détail des sujets.

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Until recently, much of the ecological theory has been based upon the unrealistic assumption of spatial and temporal homogeneity. Because environmental heterogeneity has often been viewed mainly as uninteresting noise, the need to accommodate spatial variation in the sampling designs has not been widely recognized. Accordingly, when choosing the spatial scale for a study, ecologists have tended to be highly traditional, favouring sampling devices and experimental cages of a certain size. Unfortunately, the spatial scale of conventional field techniques is often quite different from that of the organisms (Wiens 1989), and even strong aggregative responses of foragers to their resources will not be detected if an inappropriate spatial scale is chosen (Heads and Lawton 1983). Because it is often difficult to determine a priori the appropriate scale, Ives et al. (1993) strongly urged ecologists to conduct their studies, regardless of the objectives, on a wide variety of spatial scales.

Areal sampling has been the standard observation method in correlative predator–prey studies in streams. Observational plots, often stones or arbitrary areas of a stream bottom, have been sampled in either a random or systematic fashion (e.g., Hildrew and Townsend 1982; Malmqvist and Sjöström 1984; Peckarsky 1988, 1991). Data obtained with such methods are frequently irregular and distributions of counts are highly skewed. Accordingly, nonparametric statistical tests are commonly applied to detect spatial associations, if any exist, between predator and prey distributions. Dispersion indices are routinely used for examining spatial patterns and for testing for spatial randomness in these "sparsely sampled populations." Interactions between species are generally studied through association tables. A major drawback of these methods is that they operate on a single, often prefixed scale, and spatial patterns that exist outside the domain of the sampling device will not be manifested.

Analysis of Spatial Point Patterns in One- and Two-Species Systems

The second-order analysis of point processes is an efficient method for examining spatial patterns of study objects. The term "second-order" comes from the fact that this approach considers distances between pairs of points. The method provides a data-analytical summary, describing departures from randomness simultaneously on various scales. Further, asymptotical or simulation-based tests of randomness are available on any particular scale. The method can be extended for testing spatial independence in two-species systems, again on any scale.

For one-species point patterns we apply the K-statistic of Ripley (1976) (see also Ripley 1981; Getis and Franklin 1987; Andersen 1992). The K-function is defined here as

$$(1)\quad K(r) = \lambda^{-1}\,\mathrm{E}[N(r)]$$

where $N(r)$ is the number of further points (of the same species) within a circle of radius r around a randomly chosen point and λ is the point density. The expectation in (1) is conditional on the existence of a point at a fixed location. For a completely random point pattern (a spatial Poisson process) $K(r) = \pi r^2$. Here, we use the transformed version $L(r) = \sqrt{K(r)/\pi}$, which linearizes the plot of $K(r)$ versus r for a Poisson process. Then, $L(r) = r$ for all $r > 0$. Values larger than r indicate attraction (clustering, aggregation), and those smaller than r indicate repulsion (inhibition) on the scale of r. Another advantage of the L-function over the K-function is that for a completely random point pattern, the transformation $K \rightarrow L$ stabilizes the variance of the estimator. K-functions are estimated from mapped data. Points near the map boundaries constitute a serious problem, especially for small data sets. This can be addressed by using weighted estimators. Here we apply the Ohser–Stoyan edge correction (Stoyan et al. 1987). Testing for complete randomness is conducted by the asymptotic pointwise confidence bounds (Ripley 1981).

To supplement the analysis of spatial point patterns, we suggest using another second-order characteristic, the pair correlation function $g(r)$, which has the following interpretation: if x and y are two points in the study area with the interpoint distance r, and dx and dy are two infinitesimally small circles around the points, then the probability that dx and dy both contain exactly one individual will be

$$(2)\quad P = \lambda^2 g(r)\,\mathrm{d}x\,\mathrm{d}y$$

For a Poisson point pattern, $g(r) = 1$ for all $r > 0$. Values larger than 1 indicate clustering, and those smaller than 1, repulsion on the scale of r. The K- and g-functions are closely related:

$$(3)\quad K(r) = \int_0^r 2\pi u g(u)\,\mathrm{d}u$$

The g-functions can be estimated by an edge-corrected kernel estimator with the Epanechnikov kernel (see Silverman 1986; Penttinen et al. 1992). It must be emphasized that we do not use the g-function for statistical testing; it is only used as an explanatory second-order data summary, being easier to interpret than the L-function, which is applied for testing for complete randomness.

The second-order analysis of bivariate (two-species) point patterns is based on distances between pairs of points that belong to different species. The summary is the K_{12}-function (see Lotwick and Silverman 1983; Andersen 1992), which is defined as

$$(4)\quad K_{12}(r) = \lambda_2^{-1}\,N_r$$

where λ_2 is the density of type 2 points and N_r is the number of type 2 points within a distance r from an arbitrary type 1 point. If the two species occur independently of each other, then $\sqrt{K_{12}(r)} = \pi r^2$ for all $r \geq 0$. This suggests that the scaled counterpart $L_{12}(r) = \sqrt{K_{12}(r)/\pi}$ be used; this function is $= r$ for all $r > 0$, if the species are independent. K_{12}-functions are estimated by an edge-corrected estimator (Ohser–Stoyan edge correction, see Penttinen et al. 1992). A conditional Monte Carlo test (see Lotwick and Silverman 1983) is used for statistical testing of spatial independence between the two species. This test is based on toroidal random shifts of one point pattern with respect to the other; the point pattern of species 1 is fixed and that of species 2 is shifted randomly. This procedure is repeated and the K_{12}-function is estimated for each independent repetition. The upper and lower envelopes, giving the estimates of 90% pointwise confidence limits, are formed from 19 independent shifts. Again, we use the transformed version $L_{12}(r)$ for the same reasons as above.

A Case Study: Scales of Aggregation in a Lotic Predator–Prey Relationship

In many environments, food resources occur in more or less distinct patches of a certain size and shape. If these patches differ in quality, foragers should allocate their efforts predominantly to areas where they can achieve the highest rewards in terms of food intake (e.g., Pyke et al. 1977; Hassell 1978). For instance, many algal grazers in streams concentrate their foraging activity in the most profitable patches of periphyton (Hart 1981; Kohler 1984). On the other hand, predatory stream insects are apparently not able to track the spatial distribution of mobile prey (Walde and Davies 1984; Peckarsky and Penton 1985; Peckarsky 1988, 1991). Sih (1984) predicted that when both predator and prey are mobile, their spatial responses cancel one another, and no obvious interspecific pattern is observed. A positive correlation is expected only if prey are relatively immobile.

Black fly larvae are common inhabitants of fast-flowing sections in streams, where they capture seston particles from the water with their cephalic fans. They are often distributed in clusters, favoring microhabitats with suitable attachment sites and microflow patterns (e.g., Chance and Craig 1986; Ciborowski and Craig 1989; Lacoursiere 1992). The semi-sessile life-style of these insects makes them highly vulnerable to predation, and indeed, larval black flies are the most important prey item for many invertebrate predators (Feminella and Stewart 1986; Dudgeon and Richardson 1988; Muotka 1993). However, because the foraging success of many predators is severely impaired at high flow velocities, the exposed microhabitats commonly occupied by simuliid larvae may serve as partial refuges from invertebrate predation (Allan et al. 1987; Hansen et al. 1991).

In this study, we examined the spatial distribution of two invertebrate predators, *Rhyacophila obliterata* and *Isoperla grammatica*, in relation to black fly larvae (mostly *Simulium sublacustre*) and to other environmental factors in a lake outlet in northern Finland. We also analysed the spatial association between the two predators and described the

scale of association between rhyacophilids and black fly larvae. For those purposes, we developed a specific sampling plan that allows the use of efficient statistical methods developed for spatial data (see e.g., Ripley 1981; Diggle 1983; Upton and Fingleton 1985; Cressie 1991).

Material and Methods

Study Site and Species

Stream Rytipuro is an outlet of a small lake in Oulanka National Park, northern Finland (66°21′N, 29°19′E). At the study site, the stream is shallow (<20 cm), narrow (ca. 1 m), and almost without riparian canopy. The stream substrate is composed of cobble-sized stones (10–15 cm in largest diameter) with negligible amounts of moss. The stream is almost devoid of fish, the ninespine stickleback (*Pungitius pungitius*) being the only species occasionally found in the upper reaches. The main invertebrate predator, *R. obliterata*, occurs in relatively high numbers, moves sluggishly, and is large enough to be accurately observed in the field. In early summer (late May to early July), the prey assemblage is dominated by black fly larvae, which, being small and extremely numerous, cannot be individually mapped.

Sampling

The area to be mapped (a rectangle of ca. 1 m^2, hereafter called a sampling window) was selected in a rather homogeneous region of the stream bed (for habitat descriptions, see Table 1), and the locations of all predators within the sampling window were determined by measuring the Cartesian coordinates to an accuracy of 1 cm. Rhyacophilids could easily be observed through a viewing box; in contrast, isoperlids mostly occurred beneath stones, and to map their positions each stone had to be lifted separately from the stream bottom to determine whether a predator was present. Initially, we positioned a net behind a stone before lifting it, but because the stoneflies only rarely detached from the stones, we chose not to use the net. Mapping was generally completed in 2–3 h, during which time only slight changes in the positions of the predators were observed. The resultant map was used (1) to estimate densities of the predators, (2) to analyse their spatial pattern and its relation to current velocity and prey density, and (3) to assess the spatial association between the two predator species.

We marked off two sampling windows, approximately 8 m apart, and sampled the distributions of predators and black flies within them on two different occasions during the early summer of 1991. The first observations were made during the peak abundance of larval black flies (June 19 and 21, one plot each day), and the same plots were sampled again in late June–early July (June 30 and July 2), after the black flies had emerged.

On all dates except June 21 we quantified the spatial variation in flow rate and the density of black flies within the sampling windows. It is expected that stones with predators would support higher densities of black flies than arbitrarily sampled stones. To assess this, we collected two separate sets of samples from each sampling window and used them to analyse the association between rhyacophilids and black flies.

First, the number of black flies was counted on 20 randomly selected stones with at least one rhyacophilid (predator-centered samples). After the sample was taken, the stone was put back in its original position; this was done to ensure that microflow conditions within a sampling window would remain essentially the same between the two sampling occasions. Measurements of other potentially important environmental variables (current velocity, other potential prey, e.g., baetid mayflies, nemourid stoneflies, and chironomid larvae) were also made for each stone. Current was measured with a Novar Streamflo probe 403 about 1 cm above the place where the rhyacophilid was observed. Because there was no indication of predator aggregation to other prey groups (T. Muotka and A. Penttinen, unpublished; see also Muotka 1993), we focused on black flies as the major prey type in all further analyses.

Next, 20 stones were selected from arbitrary sample points (i.e., regardless of the presence of the rhyacophilid), the number of black flies and predators (if present) was counted, and measurements of other variables were made as before. Current velocity was measured approximately 1 cm above the stone surface. To obtain these latter samples we applied systematic sampling: stones were collected in regular intervals along transects perpendicular to the flow. Five such transects were established per sampling window, and four stones were collected from each transect. The sampled stones were again returned to their original locations, but the transects were shifted slightly between the two sampling occasions to ensure that the same stones were sampled only once.

Finally, we analysed the diet composition of *R. obliterata* on June 18 and July 2, as well as the diet of *I. grammatica* on June 18; insufficient numbers of *I. grammatica* were obtained on July 2. The gut contents of 25 predators of each species were placed on slides, and the entire slides were examined for identifiable animal remains. Prey items were identified, with reference to slide-mounted specimens of benthic taxa, and counted.

We believe the approach proposed here has the following advantages: (1) densities of the predators and their simuliid prey can be accurately estimated; (2) point patterns of the predators can be analysed in such a way that any possible departures and, more importantly, the scale of the departure from randomness can be detected; and (3) spatial relations between the predators, prey, and environmental parameters (e.g., current velocity) can be described.

Results

Densities of Predators, Black Flies, and Other Prey

The systematic samples were used to estimate the densities of black flies. Because the size of our sampling units (i.e., individual stones) varied considerably, we used measurements of stone length and width to define an ellipsoid approximation of the stone surface area. If observations within a sampling window are considered to be approximately independent, we have a density estimator $\lambda_{bl} = \sum m_i / \sum a_i$, where m_i is the number of individuals in the ith sample plot and a_i its area (for the variance of the estimator, see Cochran 1977, pp. 31–33). The density of black flies declined abruptly between the two sampling periods (Table 1). Pupation started around mid-June, and by June 21 almost one-third of the population had already entered the pupal stage. Emergence took place soon after, and by the beginning of July the stream was nearly devoid of black flies. In contrast, densities of other prey types were almost constant throughout the sampling period (Table 1).

TABLE 1. Depth, flow rate, stone surface area, and mean larval densities on different sampling dates (standard errors in parentheses). Areas sampled on June 19 and June 30 were the same, as were the areas sampled on June 21 and July 2.

	Sampling date			
	June 19	June 21	June 30	July 2
Depth	8.8	12.5	10.9	14.9
(cm)	(0.3)	(0.4)	(0.4)	(0.5)
Flow rate	37.3	30.1	32.2	33.2
(cm/s)	(3.6)	(4.6)	(2.3)	(4.0)
Stone area	82.5	87.9	87.9	82.5
(cm^2)	(9.4)	(7.8)	(7.8)	(9.4)
Densities (individuals/dm^2)				
R. obliterata	60.0	97.8	26.4	18.9
	(7.8)	(9.9)	(5.6)	(5.3)
I. grammatica	16.1	12.1	2.4	1.0
	(4.0)	(3.5)	(1.6)	(1.0)
Simuliidae	1096.3	—[a]	97.1	152.1
	(432.8)		(35.7)	(43.5)
Other prey	778.3	—[a]	1100.6	731.6
	(206.5)		(206.7)	(131.4)

[a]Not measured.

The systematic samples were again used to analyse black fly point patterns on the "stone scale." Because of unequal sizes of the sample plots, traditional distribution indices could not be used. Instead, a loglinear Poisson model

$$(5)\quad \ln(\mathrm{E}m_i) = \log(\lambda) + \log(a_i)$$

was fitted for testing the hypothesis of complete randomness (McCullagh and Nelder 1989). Here, m_i is the number of individuals (assumed to be Poisson distributed), $\mathrm{E}m_i$ is its expected value, λ is the density of black flies, and a_i is the stone area. On June 19, the distribution of black flies was highly aggregated. The Poisson regression applied to systematic samples gave a poor fit ($G^2 = 210.12$, df = 19, $P << 0.001$), and the remarkably large G^2/df ratio indicated overdispersion. Black flies were not counted on June 21, and their densities on the two later sampling occasions were too low for a proper analysis.

We assume the distribution of the predators in a sampling window to be a realization of a multitype spatial point process, where the points describe the locations of individuals of one or more species. Predator densities were estimated from the mapped data by $\lambda_{pred} = m/a$, i.e., the number of counts divided by the mapped area. Assuming that the number of counts was approximately Poisson distributed, we estimated the standard error of λ_{pred} using $\sqrt{m/a}$; if a spatial pattern is regular, this estimator is slightly conservative. Densities of both predators declined substantially between June 19 and 30 (Table 1). In the case of *I. grammatica*, this may partly be explained by the onset of emergence in late June – early July (see Kuusela 1984). However, the first pupae of *R. obliterata* were not observed until July 8; therefore, the decline in density of this species could not be caused by the life-cycle events. We speculate that after the emergence of black flies, rhyacophilids dispersed more randomly throughout the outlet habitat, whereas in mid-June they were mostly restricted to areas with suitable microhabitats for black fly larvae.

Point Patterns of the Predators

The point pattern of *R. obliterata* on June 19 showed a tendency towards clustering on two scales. The initial part of the *L*-function is rapidly increasing, reaching its maximal departure from the Poisson reference curve at around 4 cm, at which point the curve lies well above the 95% upper confidence bound (Fig. 1B). This shows rather strong small-scale aggregation. A tendency towards aggregation was again evidenced at the scale of 20 to 25 cm. We interpret the smaller scale as roughly corresponding to the average size of a cluster of larval black flies, whereas aggregation at the larger scale primarily indicates heterogeneity of the rhyacophilid point pattern (see Fig. 1A). The *g*-function exhibits the same pattern. Clustering at the small scale is evident and strong; and another smaller peak in the curve appears around 20 cm (Fig. 1C). On June 21, rhyacophilids were again clustered, with the tendency towards aggregation being strongest at a scale of 5–10 cm. Some clustering was observed up to a distance of 40 cm (Figs. 1D–1F). Again, the pattern on the larger scale was mainly caused by the heterogeneity in the point pattern.

In marked contrast with the spatial pattern of *R. obliterata*, the *L*-function for *I. grammatica* lies within the acceptance region of a Poisson process (Fig. 2). This point pattern (see also Fig. 1A) can be regarded as completely random. Sample size for the isoperlids on June 21 (10 individuals) was too small for pattern analysis.

At the end of June, only rhyacophilids were sufficiently abundant for analysis. Here, the *L*-function is zero and lies beneath the lower confidence bound on the scale of 0 to 5 cm, suggesting small-scale inhibition (Fig. 3B). Although this kind of spatial pattern is usually considered to be an indication of territoriality, no conclusions about the mechanism leading to the observed pattern can be made owing to the lack of direct behavioural observations of interacting individuals. The larvae appeared to form two rather loose clusters on a scale of 10 to 20 cm (Figs. 3A and 3B).

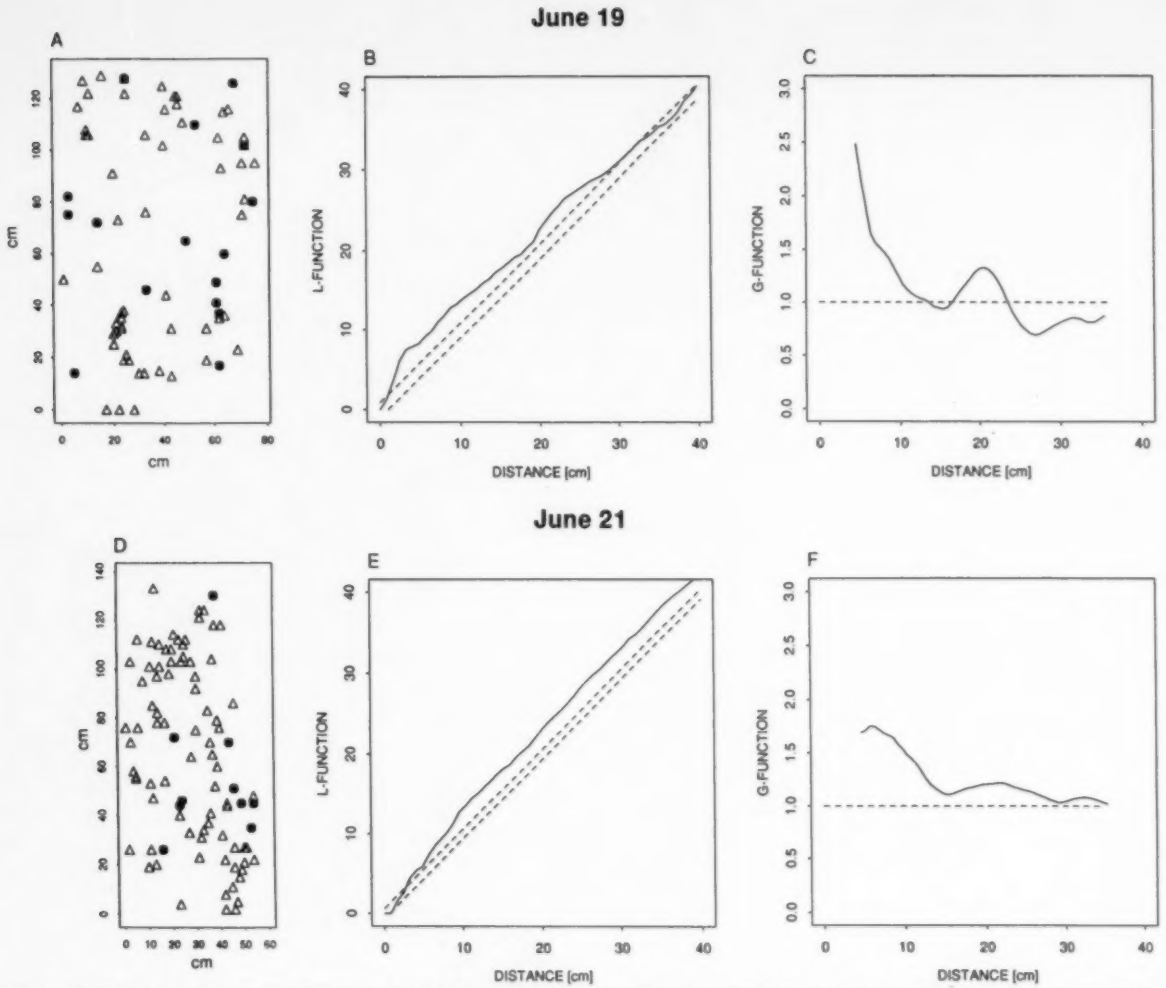

FIG. 1. Distribution maps of *R. obliterata* (△) and *I. grammatica* (●) within the sampling windows on June 19 and June 21 and the corresponding *L*- and *g*-functions for *R. obliterata*. Under complete spatial randomness, the *L*-function should lie within the asymptotic pointwise 95% confidence bounds (broken lines), and the *g*-function should be close to 1 for all distances.

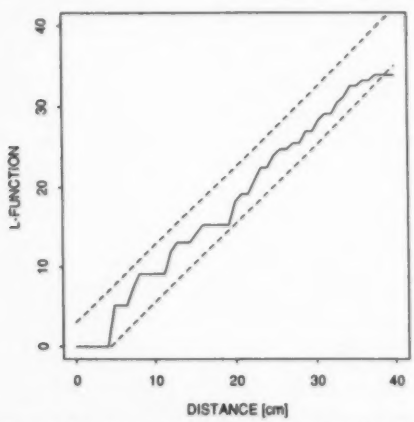

FIG. 2. The *L*-function and asymptotic pointwise 95% confidence bounds for *I. grammatica* on June 19. For the distribution map, see Fig. 1.

Interestingly, on July 2 the point pattern of *R. obliterata* was completely random (Figs. 3C and 3D).

Only data from the two earliest sampling dates could be used for examining spatial association between the predator species. On June 19, the interaction was weak, although some inhibition did occur on a scale of 10 to 20 cm (Fig. 4A). This was not unexpected, because the species only rarely co-occurred on the same stream stones. On June 21, there was no indication of spatial dependence between the two species (Fig. 4B).

Spatial Relations Between *R. obliterata* and Larval Black Flies

Association between the rhyacophilids and black flies was examined by comparing black fly densities in systematically chosen (i.e., with or without a predator) and predator-centered sample plots. Comparison of these two distributions shows whether there is an association between predator and prey on a scale related to the size of the sampling unit.

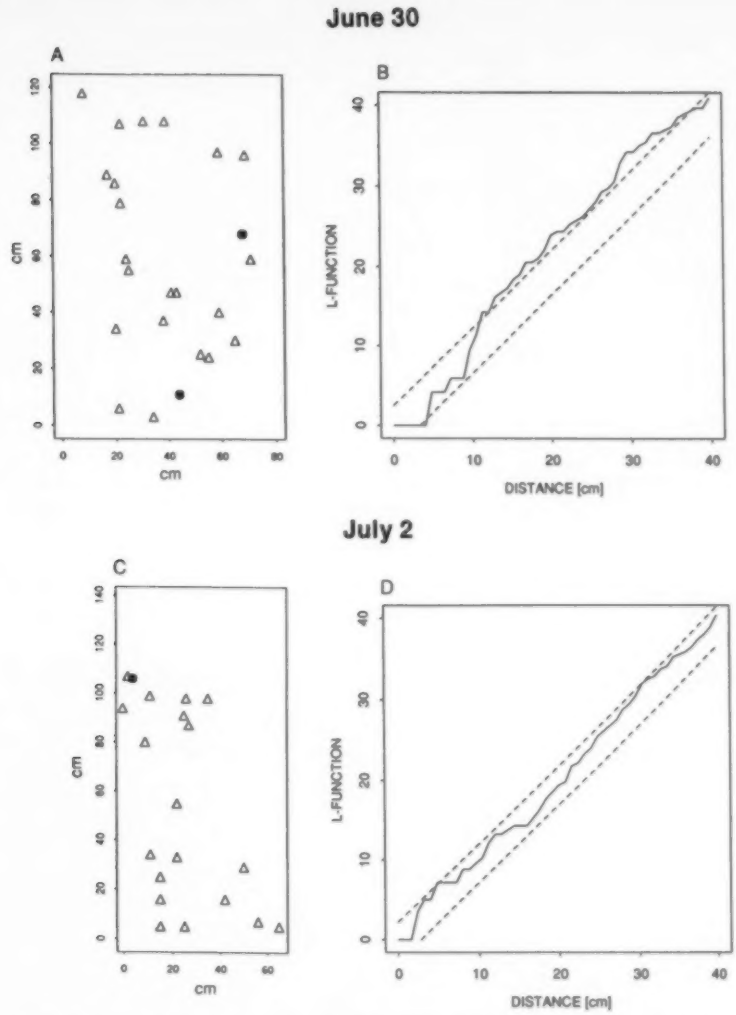

FIG. 3. Distribution maps of *R. obliterata* (△) and *I. grammatica* (●) within the sampling windows on June 30 and July 2 and the corresponding *L*-functions for *R. obliterata*.

The rationale for this is as follows: if predator and prey are independently distributed, then (from the prey point of view) an individual predator can be considered as a random location within the sampling window. In this case black fly densities do not differ among the two plot types. In the case of positive association (attraction), plots with predators are expected to have a higher density of black flies than the systematic plots. In the case of negative association (inhibition) a lower density will be found. In this study the size of the sample plots was not constant. Thus, the square roots of the observed numbers of black flies were plotted against the approximative radius of a sample plot. For a Poisson-distributed point pattern, $Em_i = \lambda\pi r^2$, where λ is the prey density; hence, the transformed counts $\sqrt{m_i}$ should lie along a straight line. Sample plots with extremely high prey density will be located well above this line. The type of these "outliers" (predator-centered or systematic samples), which is of interest here, was recorded, and the resulting contingency table (extreme vs. nonextreme prey abundance; predator-centered vs. systematic sample plot) was analysed further to detect associations between the variables.

The small-scale association between *R. obliterata* and larval black flies on June 19, the only date with an adequate amount of data, was analysed in two steps. First, the square roots of the observed counts as a function of the size (radius) of a sample plot show that 6 of the 18 predator-centered plots and only 1 of the 20 systematically sampled plots supported an exceptionally high density of simuliids (Fig. 5). Second, we constructed a 2×2 contingency table with the criteria given above. This analysis is, of course, sensitive to the subjective classification of samples being extreme or nonextreme. Therefore, we applied two different threshold densities (70 and 150 black flies/dm^2, respectively) for a sample to be classified as extreme. The χ^2-test of homogeneity showed that the rhyacophilids favoured plots with a high density of black flies in both cases ($\chi^2 = 4.97$, df = 1, $p = 0.026$; $\chi^2 = 3.62$, df = 1, $P = 0.057$, respectively). The association seemed to be the most intense at a

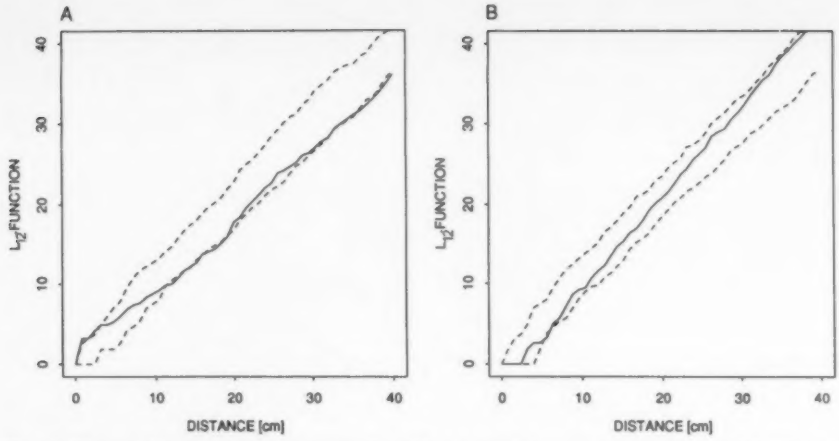

FIG. 4. The spatial association between *R. obliterata* and *I. grammatica* on June 19 (A) and June 21 (B). The L_{12}-function and its upper and lower limits (broken lines), corresponding to pointwise 90% confidence bounds, are given.

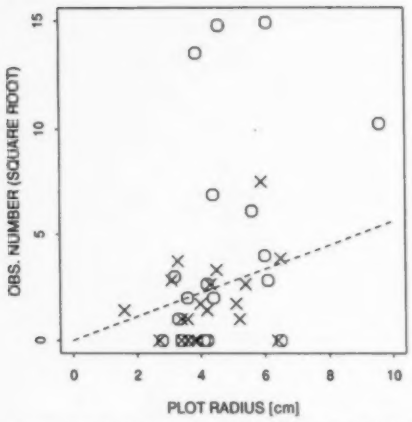

FIG. 5. Square roots of the observed numbers of larval black flies on predator-centered (○) and systematic (×) samples as a function of the size of a sample plot. The broken line ($y = 0.56x$) corresponds to the Poisson fit.

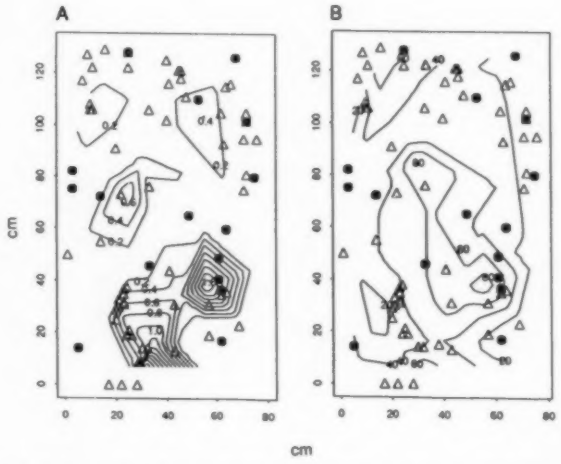

FIG. 6. Predator distributions within the sampling windows in relation to contours of (A) black fly density (10^{-4} individuals/m^2) and (B) flow rate (cm/s) on June 19. △, *R. obliterata*; ●, *I. grammatica*.

scale of 4–6 cm (Fig. 5). This means that there is a higher probability that predator-centered sample plots of this size will touch dense black fly patches than similar-sized randomly positioned sample plots.

To include yet another scale in our study, we examined spatial variation in the flow rate and in the densities of rhyacophilids and simuliids at the scale of whole sampling windows. This was achieved by overlaying the bivariate point patterns of the predators on contours of black fly density and current velocity within a sampling window. Only data from June 19 were used in this analysis. The interpolation method of Akima (1978) was applied to construct smooth surfaces from pointwise observations. Black fly larvae were concentrated in areas of high flow. Habitats of *R. obliterata* and black flies overlapped intensively in the velocity range 40–60 cm/s (Figs. 6A and 6B). *Isoperla grammatica* was mainly distributed along the margins of the sampling window, in areas where it avoided fast currents, but was at the same time spatially separated from the black flies.

Gut content analysis suggested that aggregation to microhabitats with the highest densities of black flies was associated with heavy consumption of this prey by *R. obliterata* (Table 2). The diet of *I. grammatica* on June 18 was much more varied, although larval black flies were an important food item. Chironomid larvae and a detritivorous stonefly (*Amphinemura borealis*) composed the bulk of the diet of *I. grammatica*. Interestingly, there was some indication of intra-guild predation and even cannibalism in *R. obliterata* in the beginning of July. Notwithstanding the scarcity of black flies in the benthos at this time, they still made up a major proportion of the caddisfly diet, indicating strong selection of simuliids by *R. obliterata* on the latter sampling occasion.

Discussion

Completely random point patterns of animal and plant populations are considered rare (Taylor et al. 1978; Taylor

TABLE 2. Gut contents of *R. obliterata* and *I. grammatica* as a percent of total prey consumed, by date.

	R. obliterata		*I. grammatica*	Benthos	
	June 18	July 2	June 16–18	June 18	July 2
Gut contents (%)					
Chironomidae larvae	6.1	7.1	17.1	3.3	12.9
Simuliidae larvae	51.0	23.2	29.9	47.6	12.0
Simuliidae pupae	31.6	17.9	1.5	29.6	4.5
Baetis	4.1	14.3	14.2	3.2	28.2
Heptagenia	0.0	1.8	4.9	0.3	4.8
Amphinemura	6.1	19.6	20.5	9.5	28.0
Isoperla	0.0	5.4	0.0	1.4	0.5
Rhyacophila	1.0	3.6	0.0	4.5	6.6
Other Trichoptera	0.0	7.1	11.8	0.6	2.8
No. of guts examined	25	25	25		
% of guts examined	4	24	32		
Mean no. of items/gut	4.4	2.4	1.6		
Mean predator dry wt. (mg)	12.03 (5.43)[a]	17.09 (2.08)[a]	2.79 (0.78)[a]		

[a]Standard deviation.

1984) or even unlikely ever to occur in nature (Dutilleul and Legendre 1993). Nonetheless, many recent studies have reported randomness in the spatial distribution of predaceous benthic invertebrates (Walde and Davies 1984; Peckarsky 1988, 1991; Rasmussen and Downing 1988). Mutual interference between predators has been suggested to explain the lack of aggregative responses (Peckarsky and Penton 1985). Also, the presence of fish may force invertebrate predators to avoid exposed stone surfaces (Soluk and Collins 1988; Peckarsky 1991), the microhabitat favoured by black fly larvae. In most studies, highly mobile mayfly larvae have been the major prey available for the predators. When more sedentary prey, e.g., larval black flies or cased caddis larvae, have been available, an aggregative response has been observed in predatory caddisflies (Townsend and Hildrew 1978; Malmqvist and Sjöström 1984; Muotka 1993) and an alderfly (Hildrew and Townsend 1982), but not in stoneflies (Walde and Davies 1984; Peckarsky 1988, 1991). In this study, we examined the distribution of a stonefly predator across many scales in a practically fishless stream, yet there was no evidence of a departure from randomness. We suggest that *I. grammatica* either simply avoids areas with high current velocities or becomes excluded from the best foraging sites by a species that manoeuvers more efficiently on exposed stone surfaces.

Lotic invertebrate predators have classically been regarded as highly opportunistic, changing their diet in relation to prey availability at different times of the season (e.g., Walde and Davies 1987). Our results mainly supported this conclusion; however, there was some evidence of selective feeding on simuliids by *R. obliterata*. In particular, the small-scale pattern of this predator changed from strongly aggregated to nearly completely random following the emergence of black flies. The prey assemblage in early July was dominated by species with efficient escape (e.g., *Baetis* mayflies) or avoidance (nemourid stoneflies, chironomids) antipredatory mechanisms. We suggest that in terms of prey patchiness, the outlet "landscape" was experienced as more homogeneous by the rhyacophilids once the black flies were no longer available.

Practically all natural environments are characterized by heterogeneity in space and time. Although this basic attribute of nature has long been recognized by most ecologists (McIntosh 1991), current ecological theories are "mute to scale" (Wiens 1989). There is, however, a growing demand to explicitly consider ecological patterns and processes at a wide range of spatial scales (e.g., Hurlbert 1990; Levin 1992; Milne 1992; Dutilleul and Legendre 1993; Ives et al. 1993). A concurrent development of inherently spatial approaches (e.g., landscape ecology, supply-side theory) has prompted ecologists to adopt proper analytical methods from other, traditionally more spatially oriented disciplines. Specifically, methods of geostatistics are increasingly often applied by ecologists to examine so-called "surface patterns" (Dutilleul and Legendre 1993; for a review of these methods, see Rossi et al. 1992). Another family of techniques, the second-order analysis of spatial point patterns, has also been introduced to the ecological audience by some authors (e.g., Getis and Franklin 1987; Andersen 1992). However, despite their successful application in forestry (e.g., Tomppo 1986; Penttinen et al. 1992), these efficient statistical tools have seldom been used by ecologists. We believe that the second-order analysis of mapped data applied in this study is superior to more traditional methods of spatial analysis (point-to-plant or plant-to-plant distances; quadrat sampling), because it is applicable to, and efficient in, detecting both regular and clustered patterns, whereas nearest neighbour methods are efficient for regular patterns only (i.e., in testing of small-scale inhibition). Furthermore, they are far superior to one-dimensional indices of dispersion when the pattern of a population varies from clustered at one scale to regular at another. Also, graphical display of the results serves as an efficient data summary, and Monte Carlo simulations provide a convenient way to extend statistical hypotheses testing to spatial data consisting of nonindependent observations. While mapping of aquatic insects in the field may often be a tedious process, the effort will most likely be worthwhile. This was demonstrated by the ability of the method used here to detect the spatial patterns and scales of aggregation of the two predators studied. These spatial phenomena would

not have been revealed as effectively, or at all, by more conventional sampling techniques, which strongly emphasizes the need for careful consideration of scale-related problems in studies of biotic interactions among aquatic invertebrates.

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References

AKIMA, H. 1978. A method of bivariate interpolation and smooth surface filling for irregularly distributed data points. ACM Trans. Math. Software 4: 1489–164.

ALLAN, J.D., A.S. FLECKER, AND N.L. MCCLINTOCK. 1987. Prey preference of stoneflies: sedentary vs. mobile prey. Oikos 49: 323–331.

ANDERSEN, M. 1992. Spatial analysis of two-species interaction. Oecologia 91: 134–140.

CHANCE, M.M., AND D.A. CRAIG. 1986. Hydrodynamics and behaviour of Simuliidae larvae (Diptera). Can. J. Zool. 64: 1295–1309.

CIBOROWSKI, J.J.H., AND D.A. CRAIG. 1989. Factors influencing dispersion of larval black flies (Diptera: Simuliidae): effects of current velocity and food concentration. Can. J. Fish. Aquat. Sci. 46: 1329–1341.

COCHRAN, W. 1977. Sampling techniques. John Wiley & Sons, New York. N.Y. 399 p.

CRESSIE, N. 1991. Statistics for spatial data. John Wiley & Sons, New York, N.Y. 900 p.

DIGGLE, P.J. 1983. Statistical analysis of spatial point patterns. Academic Press, London. 148 p.

DUDGEON, D., AND J.S. RICHARDSON. 1988. Dietary variations of predaceous caddisfly larvae (Trichoptera: Rhyacophilidae, Polycentropodidae and Arctopsychidae) from British Columbian streams. Hydrobiologia 160: 33–43.

DUTILLEUL, P., AND P. LEGENDRE. 1993. Spatial heterogeneity against heteroscedasticity: an ecological paradigm versus a statistical concept. Oikos 66: 152–171.

FEMINELLA, J.W., AND K.W. STEWART. 1986. Diet and predation by three leaf-associated stoneflies (Plecoptera) in an Arkansas mountain stream. Freshwater Biol. 16: 521–538.

GETIS, A., AND J. FRANKLIN. 1987. Second-order neighborhood analysis of mapped point patterns. Ecology 68: 473–477.

HANSEN, R.A., D.D. HART, AND R.A. MERZ. 1991. Flow mediates predator–prey interactions between triclad flatworms and larval black flies. Oikos 60: 187–196.

HART, D.D. 1981. Foraging and resource patchiness: field experiments with a grazing stream insect. Oikos 37: 46–52.

HASSELL, M.P. 1978. The dynamics of arthropod predator–prey systems. Monogr. Popul. Biol. 13. 237 p.

HEADS, P.A., AND J.H. LAWTON. 1983. Studies on the natural enemy complex of the holly leaf-miner: the effects of scale on detection of aggregative responses and the implications for biological control. Oikos 40: 267–276.

HILDREW, A.G., AND C.R. TOWNSEND. 1982. Predators and prey in a patchy environment: a freshwater study. J. Anim. Ecol. 51: 797–815.

HURLBERT, S.H. 1990. Spatial distribution of the montane unicorn. Oikos 58: 257–271.

IVES, A.R., P. KAREIVA, AND R. PERRY. 1993. Response of a predator to variation in prey density at three hierarchical scales: lady beetles feeding on aphids. Ecology 74: 1929–1938.

KOHLER, S.L. 1984. Search mechanism of a stream grazer in patchy environments: the role of food abundance. Oecologia 62: 209–218.

KUUSELA, K. 1984. Emergence of Plecoptera in two lotic habitats in the Oulanka National Park, northeastern Finland. Ann. Limnol. 20: 63–68.

LACOURSIERE, J.O. 1992. A laboratory study of fluid flow and microhabitat selection by larvae of *Simulium vittatum* (Diptera: Simuliidae). Can. J. Zool. 70: 582–596.

LEVIN, S.A. 1992. The problem of pattern and scale in ecology. Ecology 73: 1943–1967.

LOTWICK, H.W., AND B.W. SILVERMAN. 1983. Methods for analysing spatial processes of several types of points. J. R. Stat. Soc. B 44: 406–413.

MALMQVIST, B., AND P. SJÖSTRÖM. 1984. The microdistribution of some lotic insect predators in relation to their prey and to abiotic factors. Freshwater Biol. 14: 649–656.

MCCULLAGH, P., AND J.A. NELDER. 1989. Generalized linear models. Chapman and Hall, London. 511 p.

MCINTOSH, R.P. 1991. Concept and terminology of homogeneity and heterogeneity in ecology, p. 24–46. *In* J. Kolasa and S.T.A. Pickett [ed.] Ecological heterogeneity. Springer-Verlag, New York, N.Y.

MILNE, B.T. 1992. Spatial aggregation and neutral models in fractal landscapes. Am. Nat. 139: 32–57.

MUOTKA, T. 1993. Microhabitat use by predaceous stream insects in relation to seasonal changes in prey availability. Ann. Zool. Fenn. 30: 287–297.

PECKARSKY, B.L. 1988. Why predaceous stream insects do not aggregate with their prey? Verh. Int. Ver. Theor. Angew. Limnol. 23: 2135–2140.

PECKARSKY, B.L. 1991. Habitat selection by stream-dwelling predatory stoneflies. Can. J. Fish. Aquat. Sci. 48: 1069–1076.

PECKARSKY, B.L., AND M.A. PENTON. 1985. Is predaceous stonefly behavior affected by competition? Ecology 66: 1717–1728.

PENTTINEN, A., D. STOYAN, AND H. HENTTONEN. 1992. Marked point processes in forest statistics. For. Sci. 38: 806–824.

PYKE, G.H., H.R. PULLIAM, AND E.L. CHARNOV. 1977. Optimal foraging: a selective review of theory and tests. Q. Rev. Biol. 52: 137–154.

RASMUSSEN, J.B., AND J.B. DOWNING. 1988. The spatial response of chironomid larvae to the predator leech *Nephelopsis obscura*. Am. Nat. 131: 14–21.

RIPLEY, B.D. 1976. The second-order analysis of stationary point processes. J. Appl. Probab. 13: 225–266.

RIPLEY, B.D. 1981. Spatial statistics. John Wiley & Sons, New York, N.Y. 252 p.

ROSSI, R.E., D.J. MULLA, A.G. JOURNEL, AND E.H. FRANZ. 1992. Geostatistical tools for modelling and interpreting ecological spatial dependence. Ecol. Monogr. 62: 277–314.

SIH, A. 1984. The behavioral response race between predator and prey. Am. Nat. 123: 143–150.

SILVERMAN, B. 1986. Density estimation for statistics and data analysis. Chapman and Hall, New York, N.Y. 175 p.

SOLUK, D.A., AND N.C. COLLINS. 1988. A mechanism for interference between stream predators: responses of the stonefly *Agnetina capitata* to the presence of sculpins. Oecologia 76: 630–632.

STOYAN, D., W.S. KENDALL, AND J. MECKE. 1986. Stochastic geometry and its applications, John Wiley & Sons, Chichester. 345 p.

TAYLOR, L.R. 1984. Assessing and interpreting the spatial distribution of insect populations. Annu. Rev. Entomol. 29: 321–357.

TAYLOR, L.R., I.P. WOIWOD, AND J.N. PERRY. 1978. The density dependence of spatial behaviour and the rarity of randomness. J. Anim. Ecol. 47: 383–406.

TOMPPO, E. 1986. Models and methods for analysing spatial patterns of trees. Commun. Inst. For. Fenn. 138: 1–65.

TOWNSEND, C.R., AND A.G. HILDREW. 1978. Predation strategy and resource utilization by *Plectrocnemia conspersa* (Curtis) (Trichoptera: Polycentropodidae), p. 283–291. *In* I. Crichton. [ed.] Proceedings of the 2nd International Symposium on Trichoptera, Junk, The Hague.

UPTON, G., AND B. FINGLETON. 1985. Spatial analysis by example. Vol. 1. Point pattern and quantitative data. John Wiley & Sons, New York, N.Y. 410 p.

WALDE, S.J., AND R.W. DAVIES. 1984. The effect of intra-specific interference on *Kogotus nonus* (Plecoptera) foraging behaviour. Can. J. Zool. 62: 2221–2226.

WALDE, S.J., AND R.W. DAVIES. 1987. Spatial and temporal variation in the diet of a predaceous stonefly (Plecoptera: Perlodidae). Freshwater Biol. 17: 109–116.

WIENS, J.A. 1989. Spatial scaling in ecology. Funct. Ecol. 3: 383–397.

The Coupling of Heterotrophic Bacterial and Phytoplankton Production in a Hypertrophic, Shallow Prairie Lake

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Data from hypertrophic Humboldt Lake (Z_{max} = 6 m), Saskatchewan, support published studies indicating that bacterial numbers and production do not increase proportionally with chlorophyll concentration and primary production. There was no compensation for these relationships with increased bacterial production per cell, but our data showed an increase in production per unit bacterial biomass (273 fmol TdR·μg $C^{-1}\cdot h^{-1}$). Bacterial production (19.8–422 mg $C\cdot m^{-2}\cdot d^{-1}$) was correlated with primary production (r = 0.76), and maximum bacterial production coincided with summer cyanobacterial blooms. Water temperature was a dominant factor correlated with bacterial production (r = 0.85) and growth (r = 0.92). Depending upon the factors used to convert the rate of thymidine incorporation to gross carbon production, heterotrophic bacterial production was able to consume an average of 42% (408 mg $C\cdot m^{-2}\cdot d^{-1}$) to 67% (653 mg $C\cdot m^{-2}\cdot d^{-1}$) of plankton primary productivity. Based on these calculations, hypertrophic prairie lakes might accumulate autochthonously produced organic carbon, but this conclusion takes no account of benthic bacterial production which could be high in shallow lakes.

Les données sur le lac Humboldt un lac hypereutrophe (Z_{max} = 6 m), Saskatchewan, viennent confirmer des études indiquant que la production et les populations bactériennes ne croissent pas proportionnellement à la concentration en chlorophylle et la production primaire. On n'a pas observé de compensation, pour ces rapports, par une production bactérienne accrue par organisme, mais nos données montrent un gain de production par unité de biomasse bactérienne (273 fmol TdR · μg $C^{-1}\cdot h^{-1}$). Il existe une corrélation entre la production bactérienne (19,8–422 mg $C\cdot m^{-2}\cdot d^{-1}$) et la production primaire (r = 0,76), et la production bactérienne maximale coïncide avec les proliférations estivales de cyanobactéries. La température de l'eau constitue un facteur dominant qui est en corrélation avec la production bactérienne (r = 0,85) et la croissance (r = 0,92). Selon les facteurs appliqués à la conversion du taux d'incorporation de la thymidine dans la production brute de carbone, la production bactérienne hétérotrophique a permis la consommation d'en moyenne 42% (408 mg $C\cdot m^{-2}\cdot d^{-1}$) à 67% (653 mg $C\cdot m^{-2}\cdot d^{-1}$) de la productivité primaire planctonique. Sur la foi de ces calculs, on pense que les lacs hypereutrophes des prairies peuvent accumuler du carbone organique produit par les organismes indigènes, mais cette conclusion ne tient pas compte de la production bactérienne benthique, qui pourrait être élevée dans les lacs peu profonds.

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Scattered across the Canadian Prairies are millions of water bodies of varying size, ranging from small farm dugouts, marshes, and "pothole" lakes to large reservoirs, meltwater channel lakes, and true large lakes of >30 000 km^2, such as lakes Winnipeg and Manitoba (Barica 1987). Moreover, they span a range from fresh water to hypersaline and cover the trophic spectrum.

According to Barica (1987), most prairie lakes are naturally eutrophic because of high nutrient loading, high solar input, shallowness, internal nutrient regeneration, and extended freeze-up. Primary production in these systems arises mainly from dense blooms of algae, usually cyanobacteria (Barica 1987; Robarts et al. 1992).

Bacteria are generally accepted as the prime decomposers of organic carbon and regenerators of minerals in aquatic ecosystems. A number of studies have shown a close coupling between pelagic heterotrophic bacterial activity and growth and phytoplankton primary production, presumably associated with the production of dissolved organic carbon by the autotrophs (cf. Cole et al. 1988; White et al. 1991). It has been assumed for some time that aquatic bacterial populations are controlled by substrate availability, often existing in a semistarved or nongrowing state, and change slowly (Wright 1988). More recently, the potential importance of bacteria as a route for dissolved organic carbon to be incorporated into the classical food chain has been recognized (cf. Cole et al. 1988; White et al. 1991).

Bird and Kalff (1984) have shown that bacterial numbers in aquatic systems do not increase as rapidly as algal biomass with increasing nutrient concentrations. They suggested that this need not signify a smaller role for bacteria in enriched systems if bacterial production per unit bacterial biomass increased as total bacterial biomass increased. Estimates of bacterial production and biomass are therefore essential for assessing the importance of heterotrophic bacteria in consuming primary production and in providing organic matter

and energy for higher trophic levels in pelagic ecosystems (Kirchmann and Hoch 1988).

The factors that regulate aquatic bacterial production are a matter of considerable speculation. According to Cole et al. (1988) bacterial production is controlled by, or is directly related to, the supply of decomposable organic matter. Bacterial production in different systems should be correlated with the supply or standing stock of decomposable organic matter which, while presently impossible to quantify precisely, can be indirectly estimated by measures of variables such as primary production (Cole et al. 1988). From their review of freshwater and marine data, Cole et al. found that bacterial production for the entire water column averaged 30% of areal production. However, Robarts and Wicks (1990) reported that although bacterial production and autotrophic production were closely coupled in a hypertrophic African reservoir, bacterial production averaged only 2% of daily, areal primary production.

Bacteria have essentially been ignored in studies of prairie lakes. Barica (1987) assumed that following summer cyanobacterial blooms in prairie lakes there was a major increase in bacterial numbers and activity that depleted the water column of oxygen. In three Alberta saline lakes, Campbell and Prepas (1986) found bacterial populations ranging from 1.2×10^7 to 2.3×10^7 cells·mL^{-1}. Bacteria and detritus were concluded to be a major food resource for zooplankton in these lakes because phytoplankton biomass was insufficient to maintain the zooplankton populations. In a recent study of seasonal changes in bacterial cell volume in two Saskatchewan lakes, Tumber et al. (1993) reported that in Humboldt Lake, cell volume was most strongly associated with primary production. To our knowledge these are the only published studies on heterotrophic bacteria in prairie lakes.

The objectives of our study were: (1) to determine if bacterial production and autotrophic production were coupled in a hypertrophic, prairie lake (Humboldt Lake). The shallow (Z_{max} = 6 m) depth and frequent wind mixing of Humboldt Lake may uncouple the autotrophic–heterotrophic relationship commonly found in other aquatic systems by advecting labile dissolved organic carbon to the planktonic bacterial populations from either bottom sediments or littoral areas. In addition, the high dissolved organic carbon concentration (see below) of Humboldt Lake may also uncouple these processes if a significant portion of this material is available to bacteria. (2) To determine the ratio of areal, daily bacterial production to primary production and calculate the magnitude of organic carbon flux between these populations. (3) To assess whether bacterial production per unit bacterial biomass was high in this enriched system, where bacterial numbers would be predicted to be low relative to algal biomass, as hypothesized by Bird and Kalff (1984).

Materials and Methods

Humboldt Lake (52°09′N, 105°06′W), located in south-central Saskatchewan, is hypertrophic (maximum euphotic zone chlorophyll *a* concentration of 839 mg·m^{-3}), 6 m deep (mean depth <3 m), and slightly saline (total dissolved solids, 3.3 g·L^{-1}). Sulphate is the dominant anion (72.3%) and magnesium the dominant cation (60.7%) with concentrations of 16 084 μM and 12 754 μM, respectively (M.T. Arts, unpublished data). The lake was described in more detail by Arts et al. (1992), Robarts et al. (1992), and Tumber et al. (1993).

Water Sample Collection

Water samples were collected from a central, deep-water station with an opaque 8-L Niskin water sampler (internal diameter 7 cm; length 71.5 cm). The sampler was operated horizontally because of frequent sharp phytoplankton gradients. Water was collected from the surface at 0.5-m and then 1-m intervals to the bottom (total of eight depths). The temporal sampling intervals were: in 1989, biweekly from May to June, weekly from July to September, and again biweekly to mid-October; monthly during ice cover in January, February, and March 1990 when water samples were collected from the surface (just beneath the ice) and at 6 m; in 1990; monthly from May to October. Unstable ice conditions prevented sampling in the intervening months.

Physical and Chemical Parameters

Water temperature was measured at about midday with a Cole-Parmer thermistor, and pH was measured with a Cole-Parmer portable meter at the depth intervals given above.

Water samples taken from 0 and 6 m in Humboldt Lake were analyzed for total and dissolved nitrogen and phosphorus, dissolved (DOC) and particulate organic carbon (POC), and silica. Additional samples for NO_2 + NO_3-N and soluble reactive phosphorus (SRP) were also collected from 4 m. Chlorophyll *a* (from eight depths) was extracted in boiling (78°C) 90% ethanol and determined spectrophotometrically after correction for phaeopigments (Nusch 1980). Additional details of the field and laboratory protocols for all of the above analyses can be found in Robarts et al. (1992).

Biological Parameters

Volumetric and areal rates of phytoplankton primary production were measured using the in situ ^{14}C-light and dark bottle technique (Robarts et al. 1992).

Water samples (10 mL) were also collected from eight depths for bacterial numbers. Water was placed in sterile glass tubes and preserved with Lugol's iodine solution. Bacterial counts were done using an epifluorescence microscope and DAPI stain (Tumber et al. 1993).

Bacterial production and growth rates were calculated from the rate of [Methyl-^{3}H]thymidine (TdR) incorporation into bacterial DNA in subsamples of water collected for primary production measurements. Water samples of 10 mL were transferred to sterile screw-top glass tubes. For each sample there were two live and one control tubes. Controls were prepared by the addition of 0.5 mL 5M NaOH (Robarts and Wicks 1989).

Working solutions of TdR (2.96–3.15 TBq·nmol^{-1}, The Radiochemical Centre, Amersham, UK.) were prepared daily with sterile distilled water. TdR was added to each tube to give a final concentration of 18 nM to saturate, but not inhibit, bacterial uptake (Robarts and Zohary 1993). The tubes were attached to an incubation harness and returned to the original sampling depths. Incubation times were 15 min in summer, 30 min to 1 h in spring and fall, and up to 3 h in winter. Plots of the rate of TdR incorporation against added TdR concentration and time indicated that the TdR concentration used should have prevented isotope dilution and

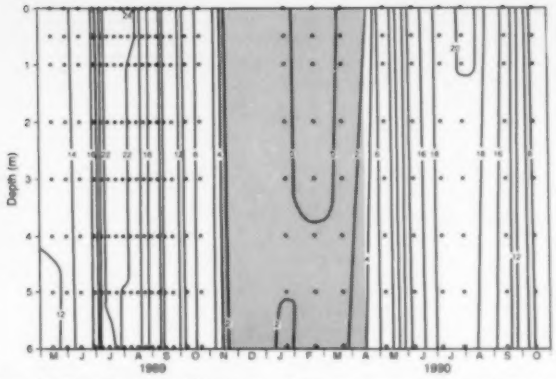

FIG. 1. Depth–time diagram of water temperature (°C) in Humboldt Lake, Saskatchewan. Shaded area indicates ice cover.

that the rate of TdR incorporation was linear over the incubation periods (Robarts and Zohary 1993).

Thymidine incorporation was stopped and the samples preserved by the addition of 0.5 mL 5M NaOH. The tubes were then stored on crushed ice until labelled DNA was extracted and purified, using phenol–chloroform and ethanol, usually within 3 h, but always within 24 h of NaOH addition to the samples. The labelled DNA was radioassayed in a liquid scintillation counter.

Bacterial production was calculated as

$$\text{Production (mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}) = 318\,\frac{\text{TdR}}{p}\,(\text{C/DNA})$$

where TdR = moles of thymidine incorporated into DNA; 318 = average molecular weight of the four nucleotides in DNA; p = proportion of thymine in DNA (average = 25); C/DNA = 6.2 for the size range of bacteria found in Humboldt Lake (Simon and Azam 1989, Table 5; Tumber et al. 1993).

Bacterial growth rates (cells·m^{-3}·h^{-1}) were calculated as production/mean carbon content per cell. We calculated the mean carbon content per cell for bacteria from 0.5 m in Humboldt Lake between May 1989 and March 1990 (Tumber et al. 1993) to be 15.68 ± 0.65 (SE) fg C·$cell^{-1}$. Specific growth rates were calculated as cells produced/bacterial numbers.

Integrated bacterial production for the euphotic zone and for the water column were obtained planimetrically from production versus depth plots.

Statistical Analyses

The data from each depth were not pooled but were analyzed (Spearman rank correlation) in eight separate categories to remove the possibility of autocorrelation with depth (Legendre and Troussellier 1988). A forward, stepwise regression (F to enter = 4.0) was used to develop a predictive relationship between daily water-column bacterial production, and physical–chemical parameters and primary productivity for Humboldt Lake.

Results

Water Temperature and Chemistry

Water temperature reached a high of 25.1°C in August 1989 and dropped to less than 0°C under the ice in winter.

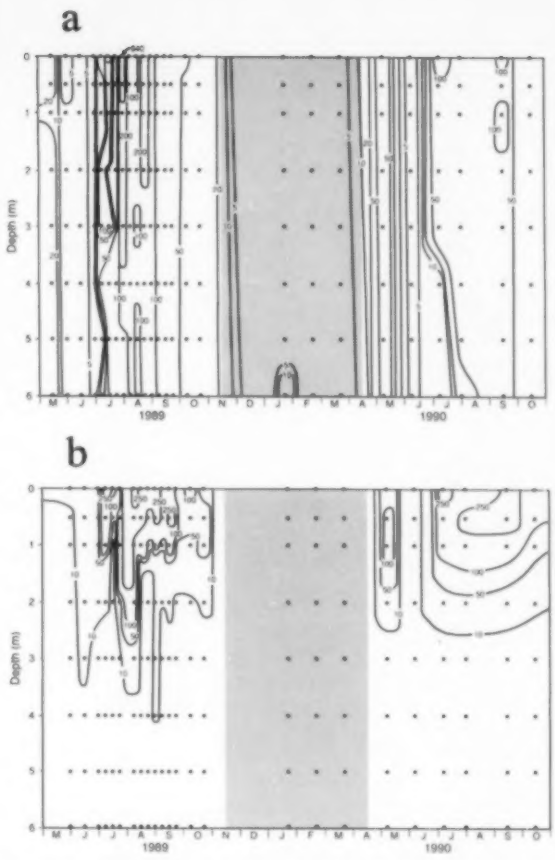

FIG. 2. Depth–time diagram of (A) chlorophyll *a* concentration (μg·L^{-1}) and (B) volumetric rate of phytoplankton primary production (mg C · m^{-3}·h^{-1}) in Humboldt Lake, Saskatchewan (Data from Robarts et al. 1992). Shaded area indicates ice cover.

The water column was markedly warmer during summer in 1989 than in 1990 (Fig. 1). Wind-generated turbulence prevented stratification of the water column during the ice-free period, although occasionally a diurnal mixed layer has been observed (Robarts et al. 1992).

Owing to runoff from surrounding agricultural lands and the historical inflow of treated sewage effluent, nutrient concentrations in Humboldt Lake are high. Soluble reactive phosphorus had an annual mean concentration of 206 μg·L^{-1}; ammonia-nitrogen (NH_3-N), a mean of 150 μg·L^{-1}; and silica, an annual mean concentration of 3630 μg·L^{-1} (Robarts et al. 1992). Nitrate (NO_3-N) plus nitrite (NO_2-N) nitrogen had a mean concentration of 32 μg·L^{-1} but dropped to <2 μg·L^{-1} in summer coincident with the onset of the summer bloom of the cyanobacterium, *Aphanizomenon flos-aquae* (Robarts et al. 1992). Additional information on the seasonal cycles of nutrients in Humboldt Lake can be found in Robarts et al. (1992).

The waters of Humboldt Lake were also characterized by high concentrations of DOC, which ranged from 19.9 to 27.9 mg·L^{-1} ($\bar{x}$ = 22.7 mg·L^{-1}).

TABLE 1. Summary of Spearman rank correlation coefficients (r) between bacterial numbers and environmental parameters in Humboldt Lake, 1989–90. Only coefficients with $p \leq 0.05$ are given; NS = $p > 0.05$, ND = parameter not determined, * = $p \leq 0.05$, ** = $p \leq 0.005$, *** = $p < 0.001$, n = 23–26.

Depth	Chlorophyll *a*	DOC	pH	TP	NO_2+NO_3	NH_3	POC	Si
Surface	NS	0.61**	NS	NS	NS	NS	NS	NS
0.5 m	NS	ND	0.66*	ND	ND	ND	ND	ND
1 m	0.47*	ND	0.64**	ND	ND	ND	ND	ND
2 m	0.45*	ND	0.66**	ND	ND	ND	ND	ND
3 m	NS	ND	0.66**	ND	ND	ND	ND	ND
4 m	NS	ND	0.67**	ND	−0.49*	ND	ND	−0.44*
5 m	0.44*	ND	0.73***	ND	ND	ND	ND	ND
6 m	0.57**	NS	0.69***	0.42*	−0.52*	−0.41*	0.57*	NS

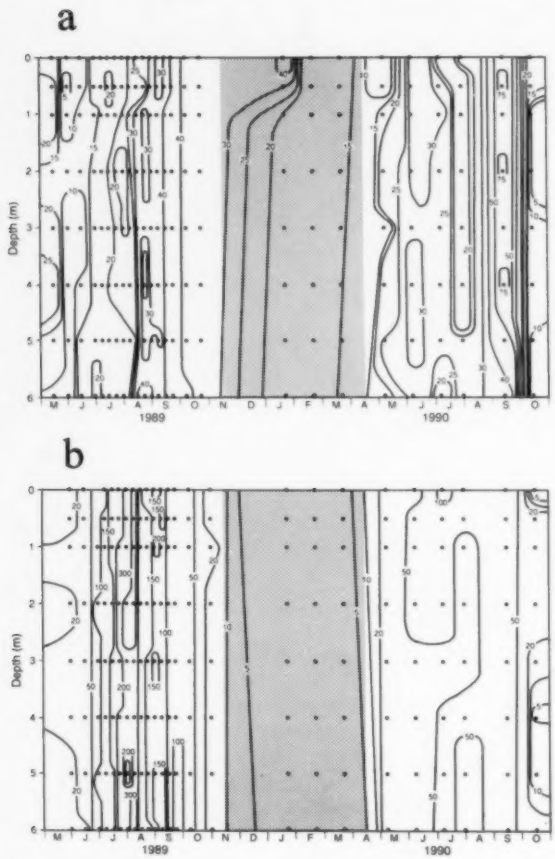

FIG. 3. Depth–time diagram of (A) bacterial numbers ($\times 10^6$ cells·mL^{-1}) and (B) heterotrophic bacterial production (pmol TdR·L^{-1}·h^{-1}) in Humboldt Lake, Saskatchewan. Shaded area indicates ice cover.

Chlorophyll *a* and Primary Production

For most of the year chlorophyll *a* was uniformly distributed through the water column (Fig. 2a) in which the mean euphotic zone depth was 3.4 m (Robarts et al. 1992). In 1989 *A. flos-aquae* began to bloom in late June and its buoyancy mechanism maintained the population at or near the surface creating a biomass gradient with depth. However, this type of seasonal pattern in water-column chlorophyll distribution generally did not occur in 1990. During the 1990 summer the phytoplankton population was dominated by *Gleothece rupestris*, a nonblooming cyanobacterium.

Primary production in Humboldt Lake was markedly stratified with depth (Fig. 2b) as a result of variations in chlorophyll concentrations (Fig. 2a) and (or) euphotic zone depth, which was largely a function of chlorophyll concentration (Robarts et al. 1992). Highest rates of production were always associated with summer cyanobacterial populations and even the fall diatom blooms produced rates in excess of 100 mg C·m^{-3}·h^{-1}. Primary production also occurred under the ice, where maximum potential rates of 0.9–5.4 mg C·m^{-3}·h^{-1} have been measured (Robarts et al. 1992).

Bacterial Numbers

Bacterial numbers in Humboldt Lake ranged from 3.5×10^6 cells·mL^{-1} in May 1989 to 110×10^6 cells·mL^{-1} in September 1990 (Fig. 3a) with a mean value of 25.62×10^6 cells·mL^{-1} for all dates and depths. The high numbers of bacteria in September were associated with a translucent material of unknown origin. Bacterial numbers under the ice were similar to levels during the open-water periods (Fig. 3a).

Correlation analysis of bacterial numbers against the environmental data revealed a consistent correlation only with pH (Table 1). Primary production was not correlated with bacterial numbers at any depth, and chlorophyll concentration was usually only weakly correlated, if at all, with bacterial numbers.

Bacterial Production and Growth

Bacterial production varied between 1.3 and 394.5 pmol TdR·L^{-1}·h^{-1} ($\bar{x}$ = 100.6 pmol TdR·L^{-1}·h^{-1}) with the lowest rates occurring in winter and the highest in summer during the cyanobacterial bloom (Fig. 3b). Production was relatively uniform throughout the water column but was markedly higher in 1989 than in 1990. When normalized to cell numbers, production ranged from 0.3×10^{-21} to 17.5×10^{-21} mol TdR·cell^{-1}·h^{-1} over the ice-free period. Specific bacterial production did not vary greatly with depth, and annual averages for each depth ranged between 4.2×10^{-21} and 5.5×10^{-21} mol TdR·cell^{-1}·h^{-1} ($\bar{x} = 4.7 \times 10^{-21}$ mol TdR·cell^{-1}·h^{-1} for all depths). The greatest values were always recorded during the cyanobacterial bloom in summer.

Bacterial production was strongly correlated with water temperature, and less strongly with chlorophyll concentration, at all depths (Table 2). Bacterial production was inversely

TABLE 2. Summary of Spearman rank correlation coefficients (r) between bacterial production (pmol TdR·L^{-1}·h^{-1}) and environmental parameters in Humboldt Lake, 1989–90. Only coefficients with $p \leq 0.05$ are given; NS = $p > 0.05$, ND = parameter not determined, * = $p \leq 0.05$, ** = $p \leq 0.005$, *** = $p < 0.001$, n = 21–25. PP = primary production (mg C·m^{-3}·h^{-1}).

Depth	Temp	Chlorophyll *a*	DOC	pH	PP	TP	$NO_2 + NO_3$	POC	PO_4-N	NH_3-N	Si
0 m	0.87***	0.76***	NS	NS	0.74***	0.64**	−0.73***	0.66**	0.50*	−0.61**	0.69***
0.5 m	0.87***	0.61*	ND	NS	0.50*	ND	ND	ND	ND	ND	ND
1 m	0.78***	0.59*	ND	NS	NS	ND	ND	ND	ND	ND	ND
2 m	0.81***	0.51*	ND	NS	NS	ND	ND	ND	ND	ND	ND
3 m	0.71***	0.66**	ND	NS	NS	ND	ND	ND	ND	ND	ND
4 m	0.78***	0.47**	ND	NS	NS	ND	−0.57*	ND	0.61**	ND	0.72**
5 m	0.74***	0.71**	ND	NS	NS	ND	ND	ND	ND	ND	ND
6 m	0.81***	0.69***	−0.47*	0.44*	0.53*	0.46*	−0.73***	0.57*	NS	−0.73***	0.63**

TABLE 3. Bacterial production and growth rate in Humboldt Lake during the ice-free periods of 1989–90, calculated from the rate of thymidine incorporation into DNA. Data given are the mean and the range.

Depth	Bacterial production (nmol TdR · m^{-3} · h^{-1})	Cell production ($\times 10^{12}$ · m^{-3} · h^{-1})	Specific growth rate (h^{-1})	Doubling time (d)	Carbon production (mg C · m^{-3} · h^{-1})
0 m	108.1	0.055	0.0026	48.4	0.87
	4.9–394.5	0.001–0.202	0.0001–0.0089	4.7–275	0.02–3.2
0.5 m	115.0	0.059	0.0029	31.8	0.92
	13.4–79.6	0.007–0.166	0.0003–0.0087	4.8–148	0.11–2.6
1 m	110.2	0.056	0.0023	31.0	0.88
	21.4–326.7	0.011–0.167	0.0004–0.0062	6.7–111	0.17–2.6
2 m	113.1	0.058	0.0026	37.8	0.91
	16.2–373.0	0.008–0.191	0.0002–0.0089	4.7–171	0.13–3.0
3 m	100.5	0.051	0.0022	41.2	0.81
	12.1–277.1	0.006–0.142	0.0002–0.0069	6.0–208	0.10–2.2
4 m	100.0	0.051	0.0022	46.2	0.80
	4.5–312.0	0.002–0.159	0.0002–0.0074	5.6–196	0.04–2.5
5 m	99.8	0.051	0.0022	41.8	0.80
	8.6–247.6	0.004–0.127	0.0002–0.0066	6.3–217	0.07–2.0
6 m	104.6	0.053	0.0023	38.5	0.84
	11.8–335.0	0.006–0.171	0.0002–0.0071	5.9–222	0.10–2.7

correlated with NO_2+, NO_3-N, and NH_3-N and positively correlated with Si concentrations at the depths at which these nutrients were measured. There were no strong correlations between bacterial production and primary production except at the surface (Table 2). This may have been due to the rapid attenuation of light in the water, which led to light-limited rates of primary production below about 0.5 m (Robarts et al. 1992). When the areal rates of primary production were substituted for the volumetric rates, bacterial production at each depth below the surface was significantly correlated with production (r = 0.48–0.59, p = 0.03–0.01).

Specific bacterial production was strongly correlated with water temperature at all depths in Humboldt Lake (r^2 = 0.78–0.90, n = 21–23, $p < 0.001$).

Volumetric rates of bacterial production as carbon were very low at all depths, ranging from 0.02 to 3.2 mg C·m^{-3}·h^{-1} with mean values of ~0.8 mg C·m^{-3}·h^{-1} (Table 3). Daily rates of bacterial production over the water column ranged from 19.8 to 422.2 mg C·m^{-2}·d^{-1} (Fig. 4). As a percentage of the equivalent rate of primary production (range 4.5–4375 mg C·m^{-2}·d^{-1}), bacterial production averaged 14.7% of phytoplankton production, with the highest value of 138% occurring in June 1990 after the decline of the spring phytoplankton bloom and when primary production was at its lowest rate. Using a bacterial growth efficiency of 35% (Schwaerter et al. 1988), the heterotrophic bacterial population of Humboldt Lake could consume an average of 42% of water column production.

Seasonal changes in the daily rate of bacterial production were correlated with water temperature (r = 0.85, $p < 0.001$), mean chlorophyll concentration of the euphotic zone (r = 0.70, p = 0.001) and the daily rate of primary production (r = 0.47, p = 0.04). A stepwise multiple regression model was generated for daily water column bacterial production:

$$\text{Production (mg C·m}^{-2}\text{·d}^{-1}) = -1566 + 11.8(\text{Temp}) + 164(\text{pH}) + 12.9(\text{Si}) \quad (r^2 = 0.85)$$

Bacterial cell production varied between 0.001×10^{12} and 0.202×10^{12} cells·m^{-3}·h^{-1} giving doubling times of 4.7–275 d during the ice-free season (Table 3). Under the ice bacterial population doubling times ranged from 477 to 887 d. The bacterial growth rate was most strongly correlated with water temperature (r = 0.92, $p < 0.001$) and Si concentration (r = 0.81, $p < 0.001$). The rate of primary production was not significantly correlated ($p > 0.2$) with bacterial growth rates.

Discussion

Comparative Bacterial Production Data

Contrary to optimistic claims in the early 1980s, there is no single, simple, and accurate method for measuring the

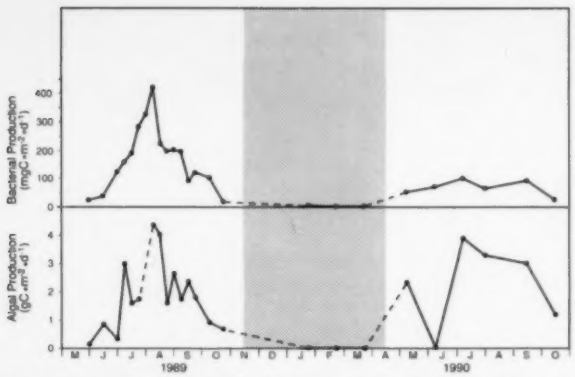

FIG. 4. Seasonal changes in heterotrophic bacterial production (mg $C \cdot m^{-2} \cdot d^{-1}$) and phytoplankton primary production (mg $C \cdot m^{-2} \cdot d^{-1}$) (Robarts et al. 1992) in hypertrophic Humboldt Lake, Saskatchewan. Shaded area indicates ice cover.

growth and production of natural bacterial populations (Robarts and Zohary 1993). Of the methods currently in use, TdR has been most frequently and widely employed. The method, however, remains controversial and the most recent assessment concluded that it is not the universal substrate required to measure bacterial production of natural populations microbial ecologists hoped it was (Robarts and Zohary 1993). Part of the problem revolves around two facts: first, not all bacteria can take up and incorporate TdR into DNA (e.g., *Pseudomonas* sp., sulphate reducing bacteria); secondly, it may not be possible to prevent intracellular dilution of the label. Our knowledge of the species composition of natural bacterial populations is very limited (Lee and Fuhrman 1991), making it impossible to assess how much variation exists in measured production rates between, and within, systems due to variations in the proportion of bacteria capable of utilizing TdR. To minimize isotope dilution, TdR is added at concentrations high enough to saturate, but not inhibit, the rate of incorporation (Robarts and Zohary 1993). Presently, the TdR assay is probably the best, relatively simple method for use with natural populations, but the measured rates should be considered as being conservative estimates of bacterial production.

Perhaps of even greater concern are the various factors used to convert moles of TdR incorporated into DNA to values of cell and carbon production (Robarts and Zohary 1993). Conversion of nmol TdR incorporated to the number of cells produced is most frequently done using an empirically derived factor. The factor is determined with water in which the natural bacterial population has been diluted with filter-sterilized water. Changes in TdR incorporation and cell number are concurrently measured over time. The most frequently measured factors are 1×10^9 – 2×10^9 cells·nmol^{-1} TdR incorporated (Robarts and Zohary 1993) but range from 0.3×10^9 to 68×10^9 cells·nmol^{-1} TdR incorporated (Moriarty 1988). The factor determined for Humboldt Lake was 2.09×10^9 cells·nmol^{-1} TdR incorporated (R.A. Snyder, U.S. EPA, Gulf Breeze, FL., unpublished data). According to Bell (1990), factors greater than about 1×10^9 – 2×10^9 cells·nmol^{-1} are due to isotope dilution. Bell also noted that it is now possible to calculate bacterial production using the growing body of data on bacterial DNA and carbon contents, as we did, instead of using empirical conversion factors. Indeed, Robarts and Zohary (1993) have argued that this method of calculating production is no less accurate than using conversion factors based on bacterial measurements from bottle enclosures of relatively long duration (≥24 h), considering the documented fact that bacterial growth sharply increases after several hours in enclosures (Ferguson et al. 1984).

Bacterial production in Humboldt Lake during the ice-free period (with a mean of about 100 nmol TdR·$m^{-3}h^{-1}$ is at the upper end of ranges published for freshwater and marine systems with the exception of some estuaries (cf. Robarts and Wicks 1990; Ducklow and Carlson 1992). Cell specific production (0.3×10^{-21} – 17.5×10^{-21} mol TdR·$cell^{-1} \cdot h^{-1}$), was within the range of values published for freshwater and marine systems (cf. Table 4, Robarts and Wicks 1990).

Several empirical models have been developed for predicting bacterial numbers and production from chlorophyll concentrations and primary production rates (Bird and Kalff 1984; Cole et al. 1988). The mean number of bacteria in Humboldt Lake during the ice-free period was 26.30×10^6 cells·mL^{-1} ($\pm 2.0 \times 10^6$ cells·mL^{-1}, 95% CL). Using the mean chlorophyll concentration, the predicted number of bacteria using Bird and Kalff's model was 23.65×10^6 cells·mL^{-1} (8.21×10^6 – 55.83×10^6 cells·mL^{-1}, 95% CL) while the Cole et al. model predicted only 9.94×10^6 cells·mL^{-1} (3.60×10^6 – 21.89×10^6 cells·mL^{-1}, 90% CL). The measured number of bacteria was higher than either model predicted, but was within the confidence limits of both models. Bird and Kalff's model was remarkably accurate in its prediction for Humboldt Lake.

Cole et al. (1988) constructed models for predicting volumetric and areal bacterial carbon production from volumetric and areal primary production, respectively. The average volumetric and areal bacterial production rates for Humboldt Lake were 32.5 mg $C \cdot m^{-3} \cdot d^{-1}$ (±3.8 mg $C \cdot m^{-3} \cdot d^{-1}$, 95% CL) and 143 mg $C \cdot m^{-2} \cdot d^{-1}$ (±44.0 mg $C \cdot m^{-2} \cdot d^{-1}$, 95% CL), respectively. The predicted values were 123 mg $C \cdot m^{-3} \cdot d^{-1}$, (15.1–415 mg $C \cdot m^{-3} \cdot d^{-1}$, 90% CL) and 370 mg $C \cdot m^{-2} \cdot d^{-1}$ (142–906 mg $C \cdot m^{-2} \cdot d^{-1}$, 90% CL). Cole et al.'s model for volumetric primary production and bacterial production rates derived solely from thymidine gave a predicted bacterial production of 148 mg $C \cdot m^{-3} \cdot d^{-1}$ (16.5–529 mg $C \cdot m^{-3} \cdot d^{-1}$, 90% CL). While measured bacterial production rates were lower than predicted rates, they fell within the broad 90% confidence bands of the models.

Cole et al.'s models were developed from literature values which were derived largely from studies using thymidine. A prime reason for the overprediction of bacterial production in Humboldt Lake could have been the various conversion factor applied in the studies used to construct the models. TdR incorporation rates were converted to cell production using factors ranging from $<1 \times 10^9$ cells·nmol^{-1} TdR to 18.3×10^9 cells·nmol^{-1} TdR. Our conversion factor was 0.51×10^9 cells·nmol^{-1} TdR (Table 3), while our empirically measured value was about four times this value. If our values in Table 3 had been calculated using the empirical factor derived for Humboldt Lake, cell production and specific growth rates would have been fourfold higher, doubling times would have been lower by the same magnitude, and carbon production would have been 1.6 times higher. These calculation differences bring the measured and

predicted bacterial carbon production rates for Humboldt Lake much closer together and well within the confidence limits of Cole et al.'s models.

The data for Humboldt Lake therefore support Bird and Kalff's (1984) and Cole et al.'s (1988) conclusions that bacterial numbers and production to not increase as rapidly as chlorophyll concentration and primary production with increasing nutrient concentration. Bird and Kalff hypothesized that this would not result in a smaller role for bacteria in enriched systems if bacterial production per unit bacterial biomass increased as total bacterial biomass increased. We found no evidence of increased bacterial production per unit bacterial cell number in Humboldt Lake. A similar conclusion was drawn by Letarte and Pinel-Alloul (1991) on the basis of data from eight Canadian and eight Danish lakes. With the exception of Letarte and Pinel-Alloul's data, data on the seasonal changes of bacterial cell volume and production for more than one lake in the same geographic area, and from one study, do not appear to have been published. We have such a 1-yr data set of hypertrophic Humboldt Lake and oligotrophic Redberry Lake (Tumber et al. 1993; R.D. Robarts, unpublished data). The average bacterial production per biomass for Humboldt Lake was 273 fmol $TdR \cdot \mu g\ C^{-1} \cdot h^{-1}$, while for the epilimnion of Redberry Lake it was 63.4 fmol $TdR \cdot \mu g\ C^{-1} \cdot h^{-1}$. These data are the first to lend support to Bird and Kalff's hypothesis that bacterial production per unit biomass increases across trophic gradients, but need confirmation from other studies. Unfortunately, Letarte and Pinel-Alloul (1991) did not give values of bacterial production per unit biomass for their lakes.

Coupling of Primary and Bacterial Production

In Humboldt Lake volumetric rates of bacterial production were strongly correlated with water temperature at all depths (Table 2). They were also correlated with chlorophyll concentration and areal primary production at all depths and with volumetric primary production, predominantly at the surface. At the depths at which nutrient concentrations were measured, they (particularly nitrogen) were significantly correlated with bacterial production. Bacterial growth rates were also most strongly correlated with water temperature ($r = 0.92$) and Si ($r = 0.81$) but not with primary production. Areal bacterial production was correlated with water temperature ($r = 0.85$), chlorophyll concentration ($r = 0.70$) and areal primary production ($r = 0.47$). Analysis of the data by year showed that the correlation between bacterial production and primary production was relatively strong ($r = 0.76$, $p < 0.001$) in 1989 but not significant in 1990 ($r = 0.35$, $p > 0.20$), owing to the high bacterial production relative to primary production during the clear-water phase in June (Fig. 4).

The significance of water temperature as a factor regulating the growth of microbial populations has received relatively little attention (Lovell and Konopka 1985) and demands further investigation, especially the way temperature interacts with, and affects, interrelationships amongst phytoplankton biomass, production, and the supply of organic carbon to bacterial populations (Pomeroy and Wiebe 1988; Pomeroy et al. 1991; Sander and Kalff 1993). Water temperature has been identified as an important regulator of bacterial production and growth either by correlative (e.g., Robarts and Wicks 1990; Findlay et al. 1991; White et al. 1991; Sander and Kalff 1993) or by empirical analysis (e.g., Robarts et al. 1991; Pomeroy et al. 1991) in a variety of aquatic systems.

In Humboldt Lake, bacterial production and bacterial cell volume (Tumber et al. 1993) appeared to be coupled to primary production. Some of the variance in the correlation between bacterial production and primary production may have been contributed by two nonphytoplanktonic autochthonous, and two allochthonous, sources of labile DOC. One autochthonous source was resuspended benthic organic matter which has been shown to stimulate heterotrophic production in coastal waters (Wainwright 1987) and was postulated by Findlay et al. (1991) to contribute organic matter to the water column of shallower portions of the Hudson River estuary. The second autochthonous source was aquatic macrophytes; the littoral zone in Humboldt Lake contains extensive macrophyte beds, especially in the shallow east and west bays of the lake. DOC released from macrophytes is a good substrate for planktonic bacteria and has been shown to be important in supporting bacterial production in other systems (Findlay et al. 1991). These autochthonous sources are supplemented by the allochthonous sources, agricultural runoff, and treated sewage effluent from the town of Humboldt, respectively.

The magnitude of the flux of carbon from phytoplankton to bacteria is usually calculated by correcting bacterial production rates for respiration using a growth yield factor. Bacterial production estimated from thymidine incorporation is assumed to equal net production in these calculations. The estimates of growth yield are widespread, ranging from <0.15 to 0.9 (Schwaerter et al. 1988). Schwaerter et al. recommended the use of a growth yield of 0.35 or less.

We used a growth yield of 0.35 and calculated that bacteria in Humboldt Lake could consume an average of 42% of phytoplankton primary production, or 408 mg $C \cdot m^{-2} \cdot d^{-1}$, assuming that all the organic carbon produced by phytoplankton was eventually available to bacteria. If we had calculated bacterial carbon production using the empirically derived conversion factor for Humboldt Lake, bacterial consumption of primary production would have averaged 67%. These calculations indicate that organic carbon production by phytoplankton in Humboldt Lake was in excess of bacterial needs and that other autochthonous and all allochthonous organic carbon sources were not required to account for the measured rates of pelagic bacterial production. Further, although the DOC concentration of Humboldt Lake was high (19.9–27.9 $mg \cdot L^{-1}$) this material was apparently not available to bacterial because bacterial numbers in Humboldt Lake were in the predicted range relative to chlorophyll concentration, as in other lakes. They also tend to support the conclusion that water temperature was a major and direct regulator of bacterial production and growth in this hypertrophic lake. White et al. (1991) concluded, from their analysis of bacterial growth and production in 57 aquatic systems, that as algal biomass rises temperature becomes increasingly important as a regulator, because at optimum levels of primary production bacteria are likely to be constrained by physical parameters, such as temperature and nutrient availability.

On the basis of our calculations, we might predict that hypertrophic prairie lakes, such as Humboldt Lake, would accumulate organic carbon. However, little information is available on organic matter content and its constituents in such lakes (Hammer 1986). Furthermore, this conclusion

takes no account of benthic bacterial production, which could be high in Humboldt Lake where all the benthic sediments are overlain by epilimnetic water. Recently, Sander and Kalff (1993) and Schallenberg and Kalff (1993) have shown that bacterial production and numbers are markedly higher in littoral sediments than in profundal sediments.

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References

ARTS, M.T., M.S. EVANS, AND R.D. ROBARTS. 1992. Seasonal patterns of total and energy reserve lipids of dominant zooplanktonic crustaceans from a hypereutrophic lake. Oecologia 90: 560–571.

BARICA, J. 1987. Water quality problems associated with high productivity of prairie lakes in Canada: a review. Water Qual. Bull. 12: 107–115, 129.

BELL, R.T. 1990. An explanation for the variability in the conversion factor deriving bacterial cell production from incorporation of [^{3}H]-thymidine. Limnol. Oceanogr. 35: 910–915.

BIRD, D.F., AND J. KALFF. 1984. Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can. J. Fish. Aquat. Sci. 41: 1015–1023.

CAMPBELL, C.E., AND E.E. PREPAS. 1986. Evaluation of factors related to the unusually low chlorophyll levels in prairie saline lakes. Can. J. Fish. Aquat. Sci. 43: 846–854.

COLE, J.J., S. FINDLAY, AND M.L. PACE. 1988. Bacterial production in fresh and salt water ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser. 43: 1–10.

DUCKLOW, H.W., AND C.A. CARLSON. 1992. Oceanic bacterial production. Adv. Microb. Ecol. 12: 113–181.

FERGUSON, R.L., E.N. BUCKLEY, AND A.V. PALUMBO. 1984. Response of marine bacterioplankton to differential filtration and confinement. Appl. Environ. Microbiol. 47: 49–55.

FINDLAY, S., M.L. PACE, D. LINTS, J.J. COLE, N.F. CARACO, AND B. PEIERLS. 1991. Weak coupling of bacterial and algal production in a heterotrophic ecosystem: the Hudson River estuary. Limnol. Oceanogr. 36: 268–278.

HAMMER, U.T. 1986. Saline lake ecosystems of the world. Dr. W. Junk Publishers, Dordrecht, The Netherlands. 616 p.

KIRCHMAN, D.L., AND M.P. HOCH. 1988. Bacterial production in the Delaware Bay estuary estimated from thymidine and leucine incorporation rates. Mar. Ecol. Prog. Ser. 45: 169–178.

LEE, S., AND J.A. FUHRMAN. 1991. Spatial and temporal variation of natural bacterioplankton assemblages studied by total genomic DNA cross-hybridization. Limnol. Oceanogr. 36: 1277–1287.

LEGENDRE, P., AND M. TROUSSELLIER. 1988. Aquatic heterotrophic bacteria: modeling in the presence of spatial autocorrelation. Limnol. Oceanogr. 33: 1055–1067.

LETARTE, Y., AND B. PINEL-ALLOUL. 1991. Relationships between bacterioplankton production and limnological variables: necessity of bacterial size considerations. Limnol. Oceanogr. 36: 1208–1216.

LOVELL, C.R., AND A. KONOPKA. 1985. The effects of temperature on bacterial production in a dimictic eutrophic lake. FEMS Microbiol. Ecol. 31: 135–140.

MORIARTY, D.J.W. 1988. Accurate conversion factors for calculating bacterial growth rates from thymidine incorporation into DNA: elusive or illusive? Ergeb. Limnol. 31: 211–217.

NUSCH, E.A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Ergeb. Limnol. 14: 14–36.

POMEROY, L.R., AND W.J. WIEBE. 1988. Energetics of microbial food webs. Hydrobiologia 159: 7–18.

POMEROY, L.R., W.J. WIEBE, D. DEIBEL, R.J. THOMPSON, G.T. ROWE, AND J.D. PAKULSKI. 1991. Bacterial responses to temperature and substrate concentration during the Newfoundland spring bloom. Mar. Ecol. Prog. Ser. 75: 143–159.

ROBARTS, R.D., M.S. EVANS, AND M.T. ARTS. 1992. Light, nutrients and water temperature as determinants of phytoplankton production in two saline, prairie lakes with high sulphate concentrations. Can. J. Fish. Aquat. Sci. 49: 2281–2290.

ROBARTS, R.D., L.M. SEPHTON, AND R.J. WICKS. 1991. Labile dissolved organic carbon and water temperature as regulators of heterotrophic bacterial activity and production in the lakes of Sub-Antarctic Marion Island. Polar. Biol. 11: 403–413.

ROBARTS, R.D., AND R.J. WICKS. 1989. [Methyl-^{3}H]thymidine macromolecular incorporation and lipid labeling: their significance to DNA labeling during measurements of aquatic bacterial growth rate. Limnol. Oceanogr. 34: 213–222.

ROBARTS, R.D., AND R.J. WICKS. 1990. Heterotrophic bacterial production and its dependence on autotrophic production in a hypertrophic African reservoir. Can. J. Fish. Aquat. Sci. 47: 1027–1037.

ROBARTS, R.D., AND T. ZOHARY, T. 1993. Fact or fiction–bacterial growth rates and production as determined by [^{3}H-methyl]thymidine? Adv. Microb. Ecol. 13: 371–425.

SANDER, B.C., AND J. KALFF. 1993. Factors controlling bacterial production in marine and freshwater sediments. Microb. Ecol. 26: 79–99.

SCHALLENBERG, M., AND J. KALFF. 1993. The ecology of sediment bacteria in lakes and comparisons with other aquatic ecosystems. Ecology 74: 919–934.

SCHWAERTER, S., M. SØNDERGAARD, B. RIEMANN, AND L.M. JENSEN. 1988. Respiration in eutrophic lakes: the contribution of bacterioplankton and bacterial growth yield. J. Plankton Res. 10: 515–531.

SIMON, M., AND F. AZAM. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51: 201–213.

TUMBER, V.P., R.D. ROBARTS, M.T. ARTS, M.S. EVANS, AND D.E. CALDWELL. 1993. The influence of environmental factors on seasonal changes in bacterial cell volume in two prairie saline lakes. Microb. Ecol. 26: 9–20.

WAINWRIGHT, S.C. 1987. Stimulation of heterotrophic microplankton production by resuspended marine sediments. Science (Wash, D.C.) 238: 1710–1712.

WHITE, P.A., J. KALFF, J.B. RASMUSSEN, AND J.M. GASOL. 1991. The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. Microb. Ecol. 21: 99–118.

WRIGHT, R.T. 1988. A model for short-term control of the bacterioplankton by substrate and grazing. Hydrobiologia 159: 111–117.

Decline of Native Unionid Bivalves in Lake St. Clair After Infestation by the Zebra Mussel, *Dreissena polymorpha*[1]

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Nalepa T.F. 1994. Decline of native unionid bivalves in Lake St. Clair after infestation by the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 51: 2227–2233.

To determine impacts of the zebra mussel, *Dreissena polymorpha*, on bivalves in Lake St. Clair, densities of Unionidae in 1990 and 1992 (after *D. polymorpha* invasion) were compared with densities in 1986 (before *D. polymorpha*). Declines in density occurred mainly at sites in the southeastern portion of the lake where unionids were highly infested with *D. polymorpha*. Unionid densities at highly infested sites declined from 2.4/m^2 in 1986, to 1.8/m^2 in 1990, and to 0/m^2 in 1992. Unionid species with light-weight shells that brood larvae over an extended period declined more between 1986 and 1990 than heavy-shelled, short-term brooders. Unionid densities at lightly infested sites located mainly in the northwestern portion of the lake did not decline: mean densities were 1.4/m^2 in 1986, 1.6/m^2 in 1990, and 1.4/m^2 in 1992. While the mean number of *D. polymorpha* per unionid was <1 at these lightly infested sites in 1990, it increased to 35 by 1992. Given this relatively slow increase in *D. polymorpha* per unionid in this portion of the lake, continued monitoring of populations should provide a better understanding of the relationship between the extent of infestations and unionid mortality.

Afin de déterminer quel effet a eu la moule zébrée *Dreissena polymorpha* sur les bivalves du lac Sainte-Claire, on a comparé la densité des unionidés en 1990 et 1992 (après l'invasion de *D. polymorpha*) à ce qu'elle était en 1986 (avant l'invasion). Il y a eu baisse de densité principalement dans les stations situées dans la partie sud-est du lac où les unionidés ont été fortement infestés par *D. polymorpha*. Dans ces stations, la densité des unionidés est passée de 2,4/m^2 en 1986 à 1,8/m^2 en 1990 et à 0/m^2 en 1992. Les espèces des unionidés à la paroi mince qui couvent leurs larves pendant longtemps ont connu un plus grand déclin entre 1986 et 1990 que celles à la coquille épaisse et qui ne couvent pas longtemps. Il n'y a pas eu abaissement de la densité des unionidés dans les stations légèrement infestées, et qui étaient situées principalement dans la partie nord-ouest du lac : la densité moyenne était de 1,4/m^2 en 1986, de 1,6/m^2 en 1990 et de 1,4/m^2 en 1992. Alors qu'il était <1 dans ces stations légèrement infestées en 1990, le nombre moyen de *D. polymorpha* par unionidé était passé à 35 en 1992. Compte tenu de cette augmentation relativement lente du nombre de *D. polymorpha* par unionidé dans cette partie du lac, on pense que le suivi des populations devrait nous permettre de mieux comprendre le rapport entre le degré d'infestation et la mortalité chez les unionidés.

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When the zebra mussel, *Dreissena polymorpha*, was first reported from North America, one of the early concerns was the impact that this species would have on bivalves of the family Unionidae (Hebert et al. 1991; Schloesser and Kovalak 1991; Mackie 1991, 1993). Unionids are filter-feeders that position themselves in substrates with their anterior end buried and their posterior end extending into the overlying water. Being a biofouler, *D. polymorpha* attaches to the exposed portion of the unionid, often reaching numbers as high as 10 000 per individual unionid (Hebert et al. 1991). Heavy infestations may interfere with normal metabolic functions of the unionid such as feeding, respiration, and reproduction and may also impede normal locomotion (Mackie 1991). To date, several surveys of unionids in the Great Lakes region have presented indirect evidence that unionid populations may be declining because of *D. polymorpha* infestations (Hunter and Bailey 1992; Haag et al. 1993). However, while these studies imply negative impacts on natural populations, quantitative surveys of unionids conducted before and after the establishment of *D. polymorpha* are presently lacking.

In this study, densities of unionids in Lake St. Clair were determined in 1990 and 1992 and compared with densities found in 1986 (Nalepa and Gauvin 1988). The 1986 survey was conducted 2 yr prior to the first discovery of *D. polymorpha* in the lake and thus provides a baseline for post-invasion comparisons.

Description of Study Site

Lake St. Clair lies at the center of the 125-km-long waterway between Lake Huron and Lake Erie. It has an area of 1100 km^2, a volume of 3.4 km^3, and a mean depth of 3 m. The lake is characterized by two distinct water masses, a northwestern mass and a southeastern mass, that contribute

[1]Contribution No. 855 to the Great Lakes Environmental Research Laboratory, Ann Arbor, Mich.

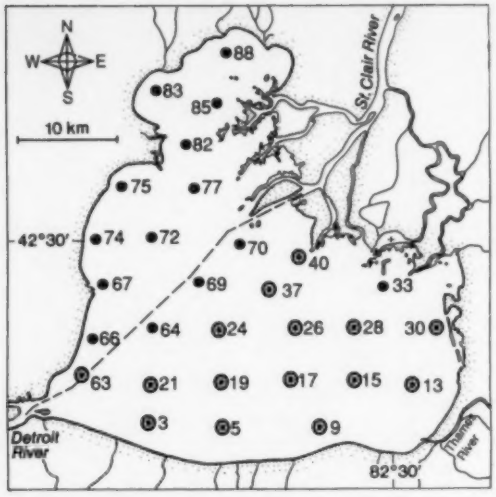

FIG. 1. Location of sampling sites in Lake St. Clair in 1986, 1990, and 1992. Circled sites indicate where unionids were highly infested with *Dreissena polymorpha* in 1990 and prior to 1990 (see text for details). Broken line indicates shipping channel.

to spatial differences in lake productivity (Leach 1972, 1980). Although the areal extent of these two water masses will vary depending on wind speed and direction, the deep channel marking the shipping lane provides a general boundary between the two water masses under the prevailing southwesterly winds (Schwab et al. 1989). A relatively high volume of water (yearly mean = 5100 m^3/s) enters the lake from Lake Huron via the St. Clair River, moves through the lake mainly along the western shoreline, and exits via the Detroit River. Inflow from the St. Clair River accounts for 98% of the water entering the lake. About 80% of the St. Clair River inflow enters the lake through distributary channels located northwest of the shipping channel (Fig. 1). This water mass is colder and contains fewer nutrients than the water mass in the southeastern portion of the lake, which is more stable, warmer, and enriched by nutrient loadings from numerous small tributaries and the Thames River. Levels of nutrients and abundances of both phytoplankton and zooplankton increase on a gradient from northwest to southeast (Leach 1972, 1980; Bricker et al. 1976). Although the two water masses are distinct, the lake is vertically well mixed, thermal stratification does not occur, and oxygen concentrations remain close to saturation throughout the year (Herdendorf et al. 1986).

Materials and Methods

Sampling sites and methods in 1990 and 1992 were the same as in 1986. Collections were made in September of each year at 29 sites located throughout the lake (Fig. 1). Site locations (longitude and latitude) were the same as given in Pugsley et al. (1985). In 1986, sites were located using Loran C, and water depth and substrate type were recorded (Table 1 in Nalepa and Gauvin 1988). Loran C was also used for positioning during the 1990 and 1992 surveys.

At each site, SCUBA divers positioned a 0.5-m^2 frame on the bottom and collected all hard material within the frame to a depth of 5 cm. The material collected included all living and dead shells of unionids, *D. polymorpha*, and hard substrate. Ten replicate quadrat samples were collected at each site; divers moved about 2–3 m between replicates to insure that each sample was taken in an undisturbed area. Immediately after collection, live unionids were opened and soft tissues placed in preweighed aluminum planchets. Any *D. polymorpha* attached to the unionids were removed and placed in 5% buffered formalin.

In the laboratory, soft tissue and shell dry weights of unionids were obtained separately after drying at 60°C for at least 48 h. Total shell lengths were determined to the nearest millimetre with vernier calipers. The relationship between dry tissue weight and shell length was described by the general allometric equation $W = aL^b$ where W is the tissue weight in grams, L is the shell length in millimetres, and a and b are constants. All *D. polymorpha* were rinsed through a screen with 500-μm openings, counted, and lengths measured using a digitizer pad (Quigley and Lang 1989). Weights (tissue and shell) of *D. polymorpha* were obtained from length–weight regressions determined from individuals collected on a monthly basis from April to November at two of the sites (stations 3 and 19) (Nalepa et al. 1993). Weights in 1990 and 1992 were determined from separate regressions. Unionid taxonomy followed that of Williams et al. (1993).

Results

On a lakewide basis, the density of unionids was significantly lower (Wilcoxon paired-sample test, $P < 0.001$, $n = 29$) in 1992 than in 1986. Mean density declined from 1.9/m^2 in 1986, to 1.7/m^2 in 1990, and to 0.7/m^2 in 1992. Unionids were not found at four sites in 1986 and 1990, but the number of sites where unionids were not found increased to 15 in 1992. In examining changes in density at individual sites, it was obvious that declines did not occur uniformly over the entire lake, but occurred mainly at sites in the southeastern portion (Fig. 2). This portion of the lake was first extensively colonized by *D. polymorpha* in 1988 (Hebert et al. 1989) and overall densities were higher here than in the northwestern portion of the lake in 1990 and 1992 (T.F. Nalepa, unpublished data; Griffiths 1993).

To determine the relationship between the decline in unionids at individual sites and the number of attached *D. polymorpha*, sampling sites were divided into two categories based on the mean number of *D. polymorpha* per unionid in 1990 : highly infested (≥55 per unionid) and lightly infested (≤3 per unionid) (Fig. 3). The actual number of *D. polymorpha* per individual unionid ranged from 0–1360 at sites in the highly infested category (97% of unionids infested) and ranged from 0–9 at sites in the lightly infested category (14% of unionids infested). The mean ratio between the total weight (shell and soft tissue) of attached *D. polymorpha* and weight of the corresponding unionid was 1.2 (range 0–8.5) at the highly infested sites. Of the 11 sites in the highly infested category, unionid densities at four of the sites were significantly lower (Wilcoxon one-tailed two-sample test, $P < 0.01$) in 1990 compared with densities in 1986 and densities at six sites were significantly lower in 1992 compared with densities in 1990 (Fig. 2). As noted, unionids were not collected at four sites in 1990 and, therefore, could not be categorized as highly infested or lightly infested. However, all of these sites were located in the

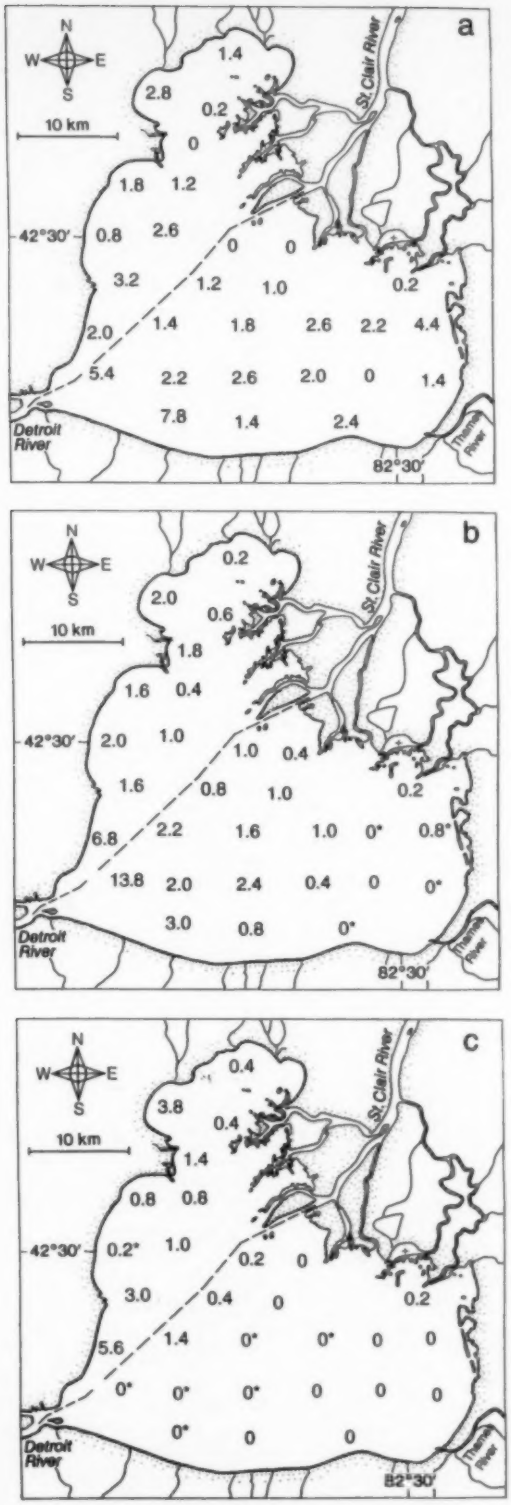

FIG. 2. Mean density (per m^2) of unionids at each of the 29 sampling sites in Lake St. Clair in 1986 (a), 1990 (b), and 1992 (c). An asterisk (*) in 1990 indicates a significant decline in density compared with 1986 and an asterisk in 1992 indicates a significant decline in density compared with 1990 (Wilcoxon one-tailed two-sample test, $P < 0.01$).

southeastern portion of the lake where unionids were highly infested prior to 1990 (see Fig. 3, Hebert et al. 1991). With these four sites included in the highly infested category, mean densities of unionids at highly infested sites (15 total) declined from 2.4/m^2 in 1986, to 1.8/m^2 in 1990, and to 0/m^2 in 1992. In contrast, unionid densities did not decline (Wilcoxon one-tailed two-sample test, $P > 0.01$) significantly at any of the 14 sites in the lightly infested category between 1986 and 1990, and declined significantly at only one site between 1990 and 1992 (Fig. 2). Mean densities at these sites were 1.4/m^2 in 1986, 1.6/m^2 in 1990, and 1.4/m^2 in 1992. Although densities of unionids did not decline at sites in the lightly infested category over the study period, the mean number of attached *D. polymorpha* per unionid increased from <1 per unionid in 1990 to 32 per unionid in 1992, and from 14% of all unionids infested to 97% infested. The total number of species declined from 18 in 1986, to 17 in 1990, and to 12 in 1992 (Table 1). In field experiments, species in the subfamilies Anodontinae and Lampsilinae were found to be more sensitive (as measured by increased mortality and loss of energy reserves) to fouling by *D. polymorpha* than species in the subfamily Ambleminae (Haag et al. 1993). To determine if differential mortality occurred in these subfamilies in Lake St. Clair over the study period, the relative frequency of anodontines and lampsilines was compared with the frequency of amblemines at both the highly infested and lightly infested sites (Table 1). At the highly-infested sites, the relative frequency of these two subfamily groups was dependent on sampling year (G-test, $P < 0.01$), with a lower proportion of anodontines and lampsilines being found in 1990 than in 1986. Species that declined the most were *Pyganodon grandis* (75%) and *Lampsilis siliquoidea* (50.6%); however, for other species such as *Leptodea fragilis* and *Lampsilis cardium* the decline was negligible (Table 1). At the lightly-infested sites, the relative frequency of subfamily groups was independent of sampling year (G-test, $P > 0.05$). With sites grouped as in 1990, relative frequencies of subfamilies in 1986 were independent of site groupings, indicating no a priori differences in spatial distributions of subfamilies.

In addition to differential mortality between subfamilies, Haag et al. (1993) found that female *L. radiata* were more sensitive to fouling by *D. polymorpha* than males. In this study, the relative frequency of female and male *L. siliquoidea* was independent of sampling year (G-test, $P > 0.01$) at both the highly infested and lightly infested sites (Table 2), indicating that differential mortality by sex did not occur. Since sublethal stress may occur prior to mortality, the relationship between soft tissue weight and shell length was determined separately for each sex at the highly infested and lightly infested sites. In unionids, this relationship has been used to assess changes in physiological state in response to varying environmental conditions (Payne and Miller 1987). Yearly trends in the dry tissue weight for a standard 60-mm individual (mean shell length of all *L. siliquoidea*) were not apparent for either sex (Table 2).

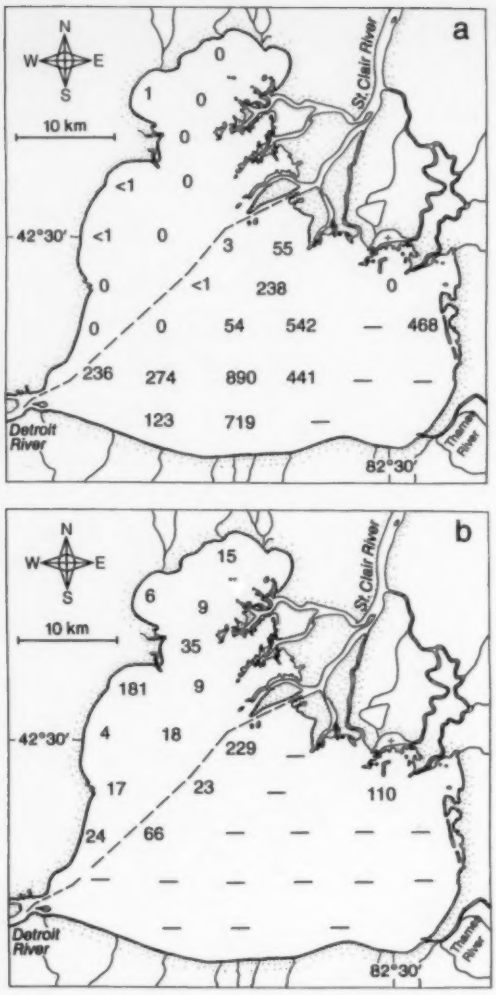

FIG. 3. Mean number of *Dreissena polymorpha* attached to unionids at each of the 29 sampling sites in Lake St. Clair in 1990 (a) and 1992 (b). *Dreissena polymorpha* was not found at any of the sites in 1986. A dash (—) indicates no unionids collected.

Discussion

In comparing temporal changes in unionid populations of Lake St. Clair in 1986 (before *D. polymorpha* invasion) and in 1990 and 1992 (after *D. polymorpha* invasion), the negative impact of *D. polymorpha* on densities of unionids was evident. At sites in the southeastern portion of the lake where unionids were first infested with *D. polymorpha* in 1988 (Hebert et al. 1991), unionid densities began to decline within 2 yr (1990), and no unionids were collected within 4 yr (1992) of infestation. At sites in the northwestern portion of the lake where unionids were not widely infested with *D. polymorpha* until after 1990, declines in densities were not evident in 1992. The delay in unionid infestations in the northwestern portion of the lake can be attributed to the high volume of water flowing into this region from the St. Clair River. This flow probably inhibited *D. polymorpha* larvae from other portions of the lake from easily colonizing this area. Also, few populations were established in the upstream reaches to serve as a source of larvae (i.e., lower Lake Huron, St. Clair River).

Since the establishment of *D. polymorpha* in North America and the subsequent finding that this species attaches to unionids in large numbers, various authors have predicted that unionids would be negatively affected over the long term (Hebert et al. 1991; Mackie 1991; Schloesser and Kovalak 1991). Others have provided only indirect evidence that natural populations of unionids were declining in areas infested with *D. polymorpha*. For instance, in sampling three sites in Lake St. Clair in 1990, Hunter and Bailey (1992) found that unionid densities were lower at a site in the southeastern portion of the lake than at a site in the southwestern portion. This spatial difference was attributed to the higher number of *D. polymorpha* per unionid at the former site. However, unionid densities tended to be lower in the southeastern portion of the lake before *D. polymorpha* became established (see densities in 1986, Fig. 2). In a survey of various sites in western Lake Erie, Haag et al. (1993) found that the ratio of live individuals to dead individuals of Anodontinae and Lampsilinae was significantly lower than the same ratio for Ambleminae. While this finding supported experimental results that showed higher mortality in the former two subfamilies when infested with *D. polymorpha*, actual declines in densities were not determined. Gillis and Mackie (1994), on the other hand, documented a decline in unionids at several sites in southwestern Lake St. Clair between 1990 and 1992.

Although unionid densities declined to $0/m^2$ between 1986 and 1992 at sites where *D. polymorpha* infestations were the greatest, it is not clear whether the entire unionid population at these sites has been eliminated. Quadrat sampling by divers is the most accurate and preferred method to quantify unionids and detect changes over time (Isom and Gooch 1986; Downing and Downing 1992), but it is not the best method to assess presence–absence because it is labor intensive and usually only a limited area of the bottom can be sampled at a given site (Kovalak et al. 1986). Other methods that assess populations over much larger areas of the bottom, such as diver observations along transects, or towing an epibenthic sled (Schloesser and Nalepa 1994), need to be used to address the specific issue of total population loss.

As noted, during the early years of the *D. polymorpha* infestation (1990 and before) in Lake St. Clair, unionids in the subfamilies Anodontinae and Lampsilinae declined to a greater extent than unionids in the subfamily Ambleminae at sites where individuals were highly infested. This result generally agrees with the short-term (3 mo) field experiments of Haag et al. (1993) in western Lake Erie. It was hypothesized that anodontines and lampsilines are more sensitive to infestations by *D. polymorpha* because they brood larvae for 9–12 mo, while amblemines brood larvae for only a few weeks to 2 mo. Long-term brooders expend more energy in the reproductive process and would be more susceptible to the unfavorable conditions created by *D. polymorpha*. Also, anodontines and lampsilines tend to have lighter shells, and the added weight of *D. polymorpha* would more likely change their center of gravity and require a greater expenditure of energy to maintain their position in the substrate. In Lake St. Clair, while overall density declines were greater in anodontines and lampsilines than in amblemines at highly infested sites, the extent of the decline varied

TABLE 1. Total number of unionids at sites where the mean number of *Dreissena polymorpha* per unionid was high (≥55) and low (≤3) in 1990. *Dreissena polymorpha* was not found in 1986. Living unionids were not found at the highly infested sites in 1992. The relative frequency of short- and long-term brooders was dependent upon sampling year (1986, 1990) at the highly infested sites (G-test, $P < 0.01$), but independent of year (1986, 1990, 1992) at the lightly infested sites.

	Highly infested (15 sites)			Lightly infested (14 sites)		
Subfamily and Species	1986	1990	1992	1986	1990	1992
Long-term brooders						
Subfamily Anodontinae						
Lasmigona complanata complanata	1	1	0	0	0	0
Pyganodon grandis	8	2	0	6	9	11
Simpsonaias ambigua	0	0	0	0	1	0
Strophitus undulatus	1	0	0	0	0	0
Subfamily Lampsilinae						
Actinonaias ligamentia	0	0	0	0	1	0
Lampsilis cardium	14	13	0	2	3	2
Lampsilis fasciola	1	0	0	0	0	0
Lampsilis siliquoidea	83	41	0	44	38	45
Leptodea fragilis	24	22	0	13	14	5
Ligumia nasuta	3	3	0	5	2	5
Ligumia recta	0	0	0	3	1	1
Obovaria subrotunda	1	2	0	0	0	0
Potamilus alatus	17	12	0	12	11	19
Truncilla donaciformis	1	0	0	0	6	1
Truncilla truncata	2	0	0	1	11	4
Total	156	96	0	86	97	94*
Short-term brooders						
Subfamily Ambleminae						
Amplema plicata plicata	2	0	0	4	0	1
Elliptio dilatata	16	24	0	4	5	3
Fusconaia flava	8	14	0	2	8	1
Quadrula quadrula	0	0	0	2	2	0
Pleurobema coccineum	0	2	0	1	0	0
Total	26	40	0	13	15	5

*Includes one unidentified young-of-the-year.

greatly across species. In contrast to the results of Haag et al. (1993) which showed greater mortality in female than in male *L. radiata*, differences in mortality between male and female *L. siliquoidea* in Lake St. Clair were not apparent.

Over the short term, mortality of infested unionids may certainly differ by subfamily, species, or even by sex, but over the long term, as found in this study, all unionids in heavily infested areas are negatively affected (i.e., densities of all individuals declined to $0/m^2$). Exact reasons for unionid mortality are not clear, but are likely related to a general decline in the unionid's physiological condition. Infested unionids display a marked, rapid decline in relative condition in just 3 mo as demonstrated by a decrease in glycogen, the main energy reserve, and an increase in stress, as measured by cellulase activity (Haag et al. 1993). Energy stores in heavily infested unionids may be depleted as a result of the following factors, either solely or in combination: (1) food is not filtered from the water effectively because of siphon blockage; (2) filtered water is already cleared of food by *D. polymorpha* or (3) maintenance of position in the substrate with the added weight of attached *D. polymorpha* leads to added metabolic costs. In the experimental treatments of Haag et al. (1993), control groups consisted of specimens of *L. radiata* with *D. polymorpha* removed. Mortality in these control groups was still 32% after 3 mo. Some mortality may have been due to handling, but this high mortality may indicate that, at least for this species, recovery from prolonged periods of depleted energy reserves does not readily occur. A loss of energy stores is likely the underlying cause of death over the long term; such a loss makes it difficult for unionids to survive periods of stress, as during the winter months. In Lake St. Clair, the greatest number of freshly dead (soft tissue still present) individuals were observed in early spring (T.F. Nalepa, personal observation). Infested unionids may also be more susceptible to the stresses of winter since they cannot effectively burrow into sediments to overwinter (Stansbery 1961; Amyot and Downing 1991).

Although *D. polymorpha* has colonized Europe for over a century and there are a number of reports of this species infesting unionids (Wagner 1936; Sebestyen 1938; Wolff 1969; Lewandowski 1976), changes in densities of unionids related to *D. polymorpha* infestations have generally not been documented. Also not documented is the long-term fate of populations in waters infested with *D. polymorpha*. In one account, Sebestyen (1938) did report large masses of dead unionids washing up on the beaches of Lake Balaton, Hungary, soon after *D. polymorpha* became established. The greatest mortalities in the lake were observed in 1935–36,

TABLE 2. Total number of individuals and tissue dry weight (g) of male and female *Lampsilis siliquoidea* at sites where the mean number of *Dreissena polymorpha* per unionid was high (≥55) and low (≤3) in 1990. The relative frequency of males and females was independent of year for both the highly infested and lightly infested sites (G-test, $P > 0.05$). Tissue dry weight is for a standard 60-mm individual as calculated from tissue dry weight – shell length regressions. No individuals were found at the highly infested sites in 1992.

	Highly infested			Lightly infested		
	1986	1990	1992	1986	1990	1992
Number collected						
Male	57	30	0	32	27	33
Female	26	11	0	12	11	12
% Female	31.3	26.8	0	27.3	28.9	26.7
Tissue dry weight						
Male	1.35	1.52	0	1.32	1.92	1.50
Female	1.70	1.71	0	1.51	1.98	1.63

about 4 yr after *D. polymorpha* was first discovered. Interestingly, the time lag between the establishment of *D. polymorpha* and greatest unionid mortality in Lake St. Clair closely paralleled that found in Lake Balaton.

Lewandowski (1976) compared the mean dry tissue weight per shell length of unionids with (mean of 20 per unionid) and without attached *D. polymorpha* from Lake Mikolajskie, Poland. There were no differences in the dry weight per shell length relation between the two groups, and this finding has been cited as evidence that unionids are not affected by *D. polymorpha* when infestations are light (Schloesser and Kovalak 1991). For unionids and molluscs in general, this relationship (or tissue weight : shell weight) has been used as an index of relative physiological condition (Russell-Hunter and Eversole 1976; Payne and Miller 1987; Williams and McMahon 1989). During periods of stress, the individual loses tissue biomass relative to the shell either through decreased tissue growth or tissue reabsorption (Russell-Hunter 1985). Although low numbers of attached *D. polymorpha* may indeed have little impact on the physiological condition of unionids, tissue dry weight per shell length did not prove to be a sensitive indicator of stress in this study; that is, there was no apparent decrease in tissue weight of *L. siliquoidea* at heavily infested sites between 1986 and 1990 even though densities declined. In Lake Mikolajskie, the mean number of *D. polymorpha* per unionid increased from 20 in 1972 to 52 in 1974 (Lewandowski 1976). This increase corresponded to a general increase in *D. polymorpha* populations during this period (Stanczykowska and Lewandowski 1993). Subsequently, by 1987, the number and biomass of unionids in Lake Mikolajskie had declined compared with 1972 (Lewandowski 1991). The decline between 1972 and 1987 was attributed to eutrophication, but *D. polymorpha* may have also played a role.

With the recent movement of *D. polymorpha* outside the Great Lakes basin into the major river systems of North America (Tucker et al. 1993), areas with unionid populations that are much more diverse and abundant than those of the Great Lakes are now threatened. Assuming *D. polymorpha* abundances will vary in these systems, an important question to be considered is how many attached *D. polymorpha* can an individual unionid tolerate before it becomes stressed and eventually dies? In southeastern Lake St. Clair, the maximum number of *D. polymorpha* per unionid increased from 100 in 1988 to a maximum of 10 000 in 1989 (Hebert et al. 1991). In 1990 at the highly infested sites in the southeastern portion of the lake, the maximum number per unionid was 1400, with a mean of 300, just prior to unionid densities decreasing to 0/m^2 in 1992. These numbers are higher than the 200 per unionid found to cause mortality and a loss of energy reserves in unionid from western Lake Erie (Haag et al. 1993). Certainly an important factor when relating the number of attached *D. polymorpha* and potential impacts on unionids is the relative "health" of populations before *D. polymorpha* becomes established. Despite a mortality event in 1985 (Neves 1987), the unionid population in Lake St. Clair prior to the introduction of *D. polymorpha* has remained generally stable over time. The number of species and their relative abundances in 1986 were little changed since a survey conducted nearly a century earlier (Nalepa and Gauvin 1988). Thus, the time period between infestations by *D. polymorpha* and observed declines in unionids in southeastern Lake St. Clair may be considered typical for a "healthy", relatively unstressed population. In western Lake Erie, where *D. polymorpha* became established at the same time as in Lake St. Clair (Griffiths et al. 1991), unionids apparently declined more rapidly than in Lake St. Clair (Schloesser and Nalepa 1994). This more rapid decline may represent the response of an already stressed population because unionids in western Lake Erie have been in a state of decline over the past few decades (Nalepa et al. 1991). However, it may also be related to the higher number of *D. polymorpha* attached to unionids in western Lake Erie than in Lake St. Clair (Schloesser and Nalepa 1994).

While the increase in number of *D. polymorpha* per unionid in southeastern Lake St. Clair was rapid, and declines in unionid densities were apparent within 2 yr of infestation, increases in the number of *D. polymorpha* per unionid in the northwestern portion of the lake will likely take place over an extended period and may never equal numbers found in the southeastern portion because of cooler temperatures and lower food supplies. Thus, continued monitoring of unionids in this portion of the lake should provide a better understanding of the relationship between the extent of *D. polymorpha* infestations and subsequent mortality in unionids.

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References

AMYOT, J.P., AND J.A. DOWNING. 1991. Endo- and epibenthic distribution of the unionid mollusc *Elliptio complanata*. J. N. Am. Benthol. Soc. 10: 280–285.

BRICKER, K.S., F.J. BRICKER, AND J.E. GANNON. 1976. Distribution and abundance of zooplankton in the U.S. waters of Lake St. Clair, 1973. J. Gt. Lakes Res. 2: 256–271.

DOWNING, J.A., AND W.L. DOWNING. 1992. Spatial aggregation, precision, and power in surveys of freshwater mussel populations. Can. J. Fish. Aquat. Sci. 49: 985–991.

GILLIS, P.L., AND G.L. MACKIE. 1994. Inpact of the zebra mussel, *Dreissena polymorpha*, on populations of unionidae (Bivalvia) in Lake St. Clair. Can. J. Zool. 72: 1260–1271.

GRIFFITHS, R.W., D.W. SCHLOESSER, J.H. LEACH, AND D.P. KOVALAK. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. Can. J. Fish. Aquat. Sci. 48: 1381–1388.

GRIFFITHS, R.W. 1993. Effects of zebra mussels (*Dreissena polymorpha*) on benthic fauna of Lake St. Clair, p. 415–437. *In* T.F. Nalepa and D.W. Schloesser [ed.]. Zebra mussels: biology, impacts, and control. Lewis Publishers/CRC Press, Boca Raton, Fla.

HAAG, W.R., D.J. BERG, D.W. GARTON, AND J.L. FARRIS. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Can. J. Fish. Aquat. Sci. 50: 13–19.

HEBERT, P.D.N., B.W. MUNCHASTER, AND G.L. MACKIE. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. 46: 1587–1591.

HEBERT, P.D.N., C.C. WILSON, M.H. MURDOCH, AND R. LAZAR. 1991. Demography and ecological impacts of the invading mollusc *Dreissena polymorpha*. Can. J. Zool. 69: 405–409.

HERDENDORF, C.E., C.N. RAPHEL, AND E. JAWORSKI. 1986. The ecology of Lake St. Clair wetlands: a community profile. U.S. Wildl. Serv. Biol. Rep. 85(7.7): 187 p.

HUNTER, R.D., AND J.F. BAILEY. 1992. *Dreissena polymorpha* (zebra mussel): colonization of soft substrata and some effects on unionid bivalves. Nautilus 106: 60–67.

ISOM, B.G., AND C. GOOCH. 1986. Rationale and sampling designs for freshwater mussels Unionidae in streams, large rivers, impoundments, and lakes, p. 46–59. *In* B.G. Isom [ed.] Rationale for sampling and interpretations of ecological data in the assessment of freshwater systems. American Society for Testing and Materials, Philadelphia, Penn.

KOVALAK, W.P., S.D. DENNIS, AND J.M. BATES. 1986. Sampling effort required to find rare species of freshwater mussels, p. 34–45. *In* B.G. Isom [ed.] Rationale for sampling and interpretations of ecological data in the assessment of freshwater systems. American Society for Testing and Materials, Philadelphia, Penn.

LEACH, J.H. 1972. Distribution of chlorophyll *a* and related variables in Ontario waters of Lake St. Clair, p. 80–86. *In* Proceedings, 15th Conference on Great Lakes Research, International Association for Great Lakes Research, Ann Arbor, Mich.

LEACH, J.H. 1980. Limnological sampling intensity in Lake St. Clair in relation to distribution of water masses. J. Gt. Lakes Res. 6: 141–145.

LEWANDOWSKI, K. 1976. Unionidae as a substratum for *Dreissena polymorpha* Pall. Pol. Arch. Hydrobiol. 23: 409–420.

LEWANDOWSKI, K. 1991. Long-term changes in the fauna of family Unionidae bivalves in the Mikolajskie Lake. Ekol. Pol. 39: 265–272.

MACKIE, G.L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia 219: 251–268.

MACKIE, G.L. 1993. Biology of the zebra mussel (*Dreissena polymorpha*) and observations of mussel colonization on unionid bivalves in Lake St. Clair of the Great Lakes, p. 153–165. *In* T.F. Nalepa and D.W. Schloesser [ed.]. Zebra mussels: biology, impacts, and control. Lewis Publishers/CRC Press, Boca Raton, Fla.

NALEPA, T.F., AND J.M. GAUVIN. 1988. Distribution, abundance, and biomass of freshwater mussels (Bivalvia: Unionidae) in Lake St. Clair. J. Gt. Lakes Res. 14: 411–419.

NALEPA, T.F., B.A. MANNY, J.C. ROTH, S.C. MOZLEY, AND D.W. SCHLOESSER. 1991. Long-term decline in freshwater mussels (Bivalvia: Unionidae) of the western basin of Lake Erie. J. Gt. Lakes Res. 17: 214–219.

NALEPA, T.F., J.F. CAVALETTO, M. FORD, W.M. GORDON, AND M. WIMMER. 1993. Seasonal and annual variation in weight and biochemical content of the zebra mussel, *Dreissena polymorpha*, in Lake St. Clair. J. Gt. Lakes Res. 19: 541–552.

NEVES, R.J. 1987. Recent die-offs of freshwater mussels in the United States: an overview, p. 7–18. *In* R.J. Neves [ed.] Proceedings of the workshop on die-offs of freshwater mussels in the United States. U.S. Fish. and Wildl. Service, Upper Mississippi River Conservation Committee, Rock Island, Ill.

PAYNE, B.S., AND A.C. MILLER. 1987. Effects of current velocity on the freshwater bivalve *Fusconaia ebena*. Am. Malacol. Bull. 5: 177–179.

PUGSLEY, C.W., P.D.N. HEBERT, G.W. WOOD, G. BROTEA, AND T.W. OBAL. 1985. Distribution of contaminants in clams and sediments from the Huron–Erie corridor. I. PCB's and octachlorostyrene. J. Gt. Lakes Res. 11: 275–289.

QUIGLEY, M.A., AND G.L. LANG. 1989. Measurement of amphipod body length using a digitizer. Hydrobiologia 171: 255–258.

RUSSELL-HUNTER, W.D. 1985. Physiological, ecological and evolutionary aspects of molluscan tissue degrowth. Am. Malacol. Bull. 3: 213–221.

RUSSELL-HUNTER, W.D., AND A.G. EVERSOLE. 1976. Evidence for tissue degrowth in starved freshwater pulmonate snails (*Helisoma trivolis*) from tissue, carbon, and nitrogen analysis. Comp. Biochem. Physiol. 54A: 447–453.

SCHLOESSER, D.W., AND W. KOVALAK. 1991. Infestation of unionids by *Dreissena polymorpha* in a power plant canal in Lake Erie. J. Shellfish Res. 10: 355–359.

SCHLOESSER, D.W., AND T.F. NALEPA. 1994. Dramatic decline of unionid bivalves in offshore waters of western Lake Erie after infestation by the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 51: 2234–2242.

SCHWAB, D.J., A.H. CLITES, C.R. MURTHY, J.E. SANDALL, L.A. MEADOWS, AND G.A. MEADOWS. 1989. The effect of wind on transport and circulation in Lake St. Clair. J. Geophys. Res. 94: 4947–4958.

SEBESTYEN. O. 1938. Colonization of two new fauna-elements of Pontus-origin (*Dreissena polymorpha* Pall. and *Corophium curvispinum* G. O. Sars forma devium Wundsch) in Lake Balaton. Verh. Internat. Ver. Theor. Angew. Limnol. 8: 169–182.

STANCZYKOWSKA, A., AND K. LEWANDOWSKI. 1993. Thirty years of *Dreissena polymorpha* ecology in Mazurian Lakes of northeastern Poland, p. 3–37. *In* T.F. Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis Publishers/CRC Press, Boca Raton, Fla.

STANSBERY, D.H. 1961. The naiades (Mollusca, Pelecypoda, Unionacea) of Fishery Bay, South Bass Island, Lake Erie. Part 1. Introduction, history, faunal origins, and physiography. Sterkania 5: 1–37.

TUCKER, J.K., C.H. THEILING, K.D. BLODGETT, AND P.A. THIEL. 1993. Initial occurrences of zebra mussels (*Dreissena polymorpha*) on freshwater mussels (Family Unionidae) in the upper Mississippi River system. J. Freshwater Ecol. 8: 245–251.

WAGNER, N. 1936. Die wandermuschel (*Dreissena*) erobert den Plattensee. Natur Volk 66: 37–41.

WILLIAMS, C.J., AND R.F. MCMAHON. 1989. Annual variation of tissue biomass and carbon and nitrogen content in the freshwater bivalve *Corbicula fluminea* relative to downstream dispersal. Can. J. Zool. 67: 82–90.

WILLIAMS, J.D., M.L. WARREN, JR., K.S. CUMMINGS, J.L. HARRIS, AND R.J. NEVES. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18: 6–22.

WOLFF, W.J. 1969. The molluscs of the estuarine region of the Rivers Rhine, Meuse, and Scheldt in relation to the hydrography of the area. II. The Dreissenidae. Basteria 33: 93–112.

Dramatic Decline of Unionid Bivalves in Offshore Waters of Western Lake Erie After Infestation by the Zebra Mussel, *Dreissena polymorpha*

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Schloesser, D.W., and T.F. Nalepa. 1994. Dramatic decline of unionid bivalves in offshore waters of western Lake Erie after infestation by the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 51: 2234–2242.

Unionid bivalves and attached epizoic zebra mussels (*Dreissena polymorpha*) were collected at one index station in 1989, 1990, and 1991 and at 17 stations in 1991 in offshore waters of western Lake Erie of the Laurentian Great Lakes. Sampling at the index station revealed that the proportion of live unionids declined from 53% in September 1989 to 17% in May–June 1990 and to 0% in September 1990: this 100% mortality coincided with heavy infestation by zebra mussels. Quantitative sampling with a Ponar grab at the 17 stations in 1991 revealed a widespread and dramatic reduction in unionid populations. In 1982, five unionid species occurred at 35% of the stations at a density of 4/m^2, whereas in 1991, no live unionid species were found. Qualitative sampling with an epibenthic sled at the 17 stations in 1991 yielded only 4 live specimens of 2 species (*Amblema plicata plicata* and *Fusconaia flava*) and 187 dead specimens of 10 species. These and other results indicate that unionid populations are being negatively affected by zebra mussels in the Great Lakes. Similar impacts on unionids are expected to occur where zebra mussels become abundant throughout North America.

Des bivalves unionidés et des moules zébrées épizoïques (*Dreissena polymorpha*) ont été prélevés à une station de référence en 1989, 1990 et 1991, ainsi qu'à 17 stations en 1991 au large dans les eaux de la partie ouest du lac Érié, l'un des Grands Lacs laurentiens. L'échantillonnage de la station de référence a révélé que la proportion des unionidés vivants est passée de 53% en septembre 1989 à 17% en mai–juin 1990 et à 0% en septembre 1990. Cette mortalité complète coïncidait avec une intense infestation par des moules zébrées. L'échantillonnage quantitatif au moyen d'une benne preneuse du type Ponar à 17 stations, en 1991, a mis en évidence une réduction à la fois étalée et spectaculaire des populations d'unionidés. En 1982, cinq de ces espèces étaient observées à 35% des stations en densité de 4/m^2, alors qu'en 1991, aucun sujet vivant de ces espèces n'était trouvé. L'échantillonnage qualitatif au moyen d'un traîneau épibenthique dans les 17 stations, en 1991, n'a rapporté que 4 spécimens vivants de 2 espèces (*Amblema plicata plicata* et *Fusconaia flava*), et 187 spécimens morts qui appartenaient à 10 espèces. Ces résultats, comme d'autres, indiquent que les populations d'unionidés souffrent de la présence de la moule zébrée dans les Grands Lacs. On pense que des effets similaires sur les unionidés devraient se produire lorsque la moule zébrée deviendra abondante partout en Amérique du Nord.

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Invasion of the zebra mussel, *Dreissena polymorpha* (Bivalvia: Dreissenidae), into the Laurentian Great Lakes is of great concern because, at high densities, mussels may negatively affect raw water users and indigenous fauna and flora (Griffiths et al. 1989; Hebert et al. 1989; Gannon et al. 1990; Schloesser et al. 1990; Sblendorio et al. 1991). In 1989, zebra mussel populations in the Great Lakes increased rapidly and reached maximum densities (> 700 000 / m^2) far greater than those recorded in European waters (reviewed in Nalepa and Schloesser 1993). At present, it is not known if zebra mussels will reach high densities in all waters that they invade, but they are expected to spread throughout much of North America during the next 20 yr (Griffiths et al. 1989, 1991; Strayer 1991; Neary and Leach 1992).

The epizoic colonization of hard-bodied invertebrates including snails, crayfish, and unionid bivalves by zebra mussels has been an observed characteristic of the zebra mussel invasion in the Laurentian Great Lakes (Nalepa and Schloesser 1993). In Europe, zebra mussels are especially epizoic on unionid bivalves (Lewandowski 1976), where they attach to shells by byssal structures (Fig. 1). Infestation of unionids by zebra mussels in the Great Lakes has been observed in nearshore waters of Lake St. Clair (Hebert et al. 1989, 1991; Mackie 1991, 1993; Hunter and Bailey 1992; Nalepa 1994; Gillis and Mackie 1994), western Lake Erie (Schloesser and Kovalak 1991; Garton and Haag 1991; Haag et al. 1993), eastern Lake Erie (D.W. Schloesser, personal observation), Lake Huron (T.F. Nalepa, personal observation), and Lake Michigan (E. Marsden, Illinois Natural History

Survey, Zion, IL 60099, personal observation). There is concern, but little confirmatory evidence, about detrimental effects of infestation on naturally occurring unionid populations (reviewed in Schloesser and Kovalak 1991).

The present study was conducted to assess the effects of zebra mussels on unionids in offshore waters of western Lake Erie. This information should be valuable in determining the potential effects of zebra mussel infestations on unionids elsewhere in North America.

Methods

Unionid bivalves and infesting zebra mussels were collected in offshore waters of western Lake Erie at one index station (9L) in September 1989, May–June and September 1990, and July 1991, and at 17 stations in June–July 1991; all 17 stations were previously sampled for unionid bivalves in 1961, 1972, and 1982 (Fig. 2; Carr and Hiltunen 1965).

Qualitative sampling was performed with an epibenthic sled (46 × 25 cm) at the index station (9L) during all four sampling periods (Fig. 2). The sled was similar to that used by Miller et al. (1989). The sled was repetitively pulled (ca. 5 min per tow) with a small boat for about the same accumulative amount of time (i.e., 4 h) each sampling period. A minimum 3:1 ratio of tow rope length to water depth was maintained, and the rope was manually monitored to ensure contact of the sled with substrates during sampling. Sediments were composed of soft, pudding-like mud under a thin layer (ca. 1 cm) of silt. Unionids and attached zebra mussels were removed from the sled, individually preserved in a 5% buffered ($CaCO_3$) formalin solution, and taken to the laboratory.

Qualitative and quantitative sampling were performed at the 17 stations located throughout western Lake Erie in June–July 1991 (Fig. 2). At each station, one epibenthic sled tow (qualitative, 5 min per station) was performed as described above. In addition, three replicate Ponar grab (quantitative, 0.05 m^2) samples were collected at each station to compare with the published literature (Nalepa et al. 1991). Sediments consisted primarily of soft mud, which filled 100% of the Ponar at all stations except 4R, 5R, 5M, and 4L, where sediments consisted of a combination of silt, mud, and sand, which filled between 1/2 and 1/8 of a Ponar sample. Samples were washed in a U.S. Standard No. 30 sieve (0.65mm mesh), individually preserved in a 5% buffered ($CaCO_3$) formalin solution, and taken to the laboratory.

In the laboratory, samples were washed over a U.S. Standard No. 60 sieve (0.25-mm mesh). Unionid shells were removed from debris. Individual half shells of dead unionids that could be paired were included in the present study and unpaired half shells were discarded. Unionids and attached zebra mussels were identified, counted, measured, and weighed. Unionids were identified following Clarke (1981) and by comparisons with bivalve taxonomic reference collections. Taxonomic nomenclature follows Turgeon et al. (1988) and Williams et al. (1993) with the exception of combining *Lampsilis siliquoidea* (Barnes, 1823) and *L. radiata radiata* (Gmelin, 1791) as *L. siliquoidea* because these two species interbreed (Clarke 1981). Dry weights (105°C for 48 h) of individual unionids and infesting mussels <6-mm long and whole millimetre size categories >6 mm (i.e., 6 mm, 7 mm, ...) long were determined. Length–frequency distributions were constructed from shell length measurements

FIG. 1. The zebra mussel, *Dreissena polymorpha*, showing the ventrally located byssal structures that enable this species to be epizoic on unionid bivalves.

in 1-mm size classes. A randomly selected subsample of 200–300 mussels <6-mm long per unionid and all mussels >7-mm long were measured for each sampling period. Length–frequency distributions of unmeasured mussels <6 mm were based on the proportions of measured subsampled mussels in each whole millimetre size group. Cohort groups were based on lengths corresponding to the smallest number of mussels between length–frequency distribution peaks. Age determinations of young-of-the-year and young-of-the-year-plus mussels were based on a January 1 spawn date (Griffiths et al. 1991; Schloesser and Kovalak 1991). This procedure is adequate to separate annual size groups of mussels during the sampling period in the study area (Schloesser and Kovalak 1991). Infestation is used to describe the colonization of unionids by zebra mussels following the concept that mussels are ectoparasites as described by Margolis et al. (1982), used by Schloesser and Kovalak (1991), and demonstrated in the present study.

Results

Index Station

Total proportions of live and dead unionids changed dramatically at the index station between September 1989 and July 1991 (Fig. 3). In September 1989, the unionid collection consisted of 53% live individuals (of 54 total individuals); in May–June 1990, the population was 17% live (54 individuals). In September 1990 (56 individuals) and July 1991 (46 individuals) no live unionids were collected.

Eight unionid species were collected. These taxa, and the percent composition of the total number of individuals (210) include: *Lampsilis siliquoidea* (65%), *Ligumia nasuta* (20%), *Leptodea fragilis* (6%), *Pyganodon grandis* (4%), *Potamilus*

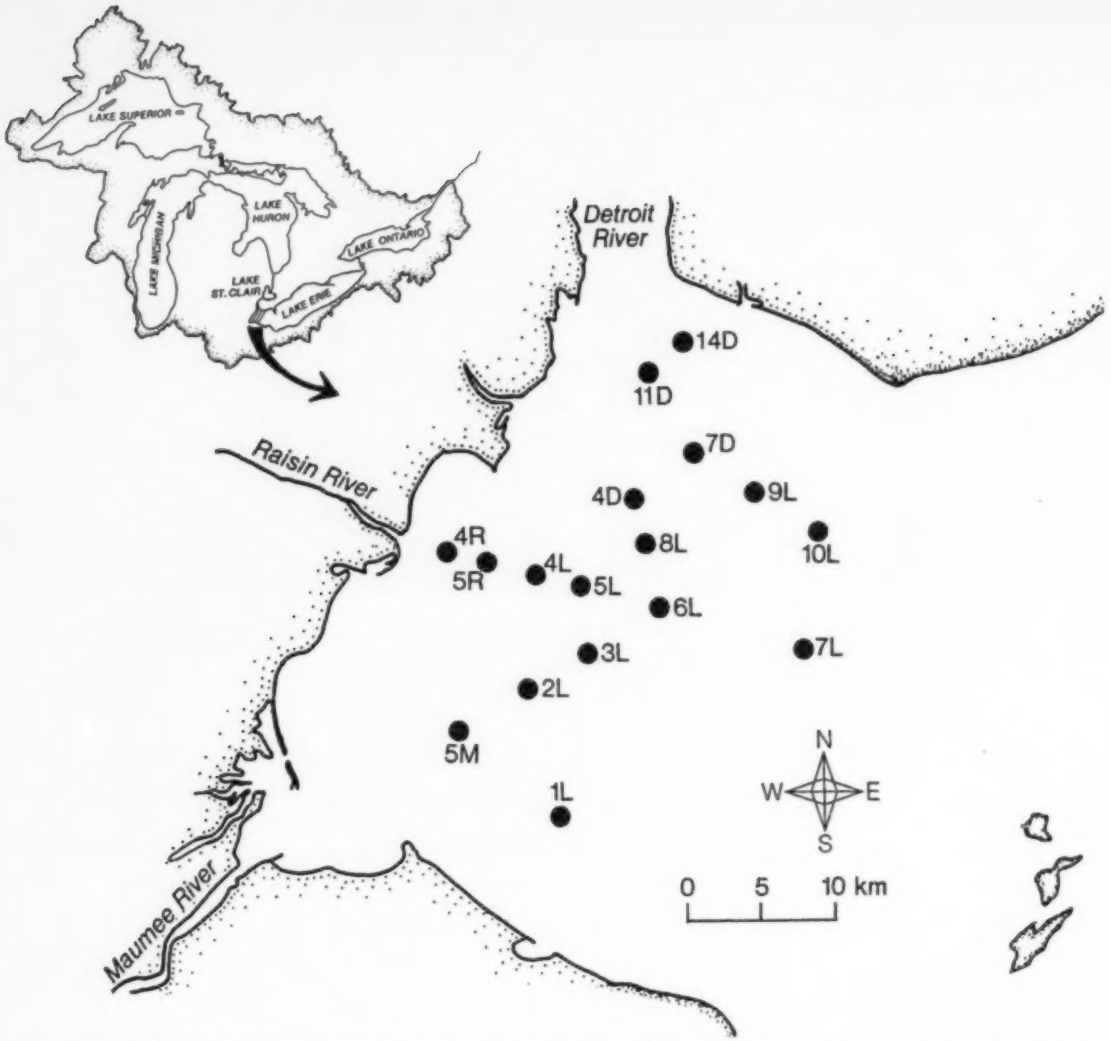

FIG. 2. Locations of one index station (9L) and 17 stations where unionid bivalves and infesting zebra mussels (*Dreissena polymorpha*) were collected in western Lake Erie 1989–91.

alatus (2%), *Lampsilis cardium* (1%), *Amblema plicata plicata* (1%), and *Elliptio dilatata* (<1%). The only consistently collected species was *L. siliquoidea*, which accounted for the majority of changes of live (89% of the total number) and dead (60% of the total number) individuals.

All live unionids were infested by zebra mussels (Table 1). Proportions of dead unionids showing infestation declined from 77% in September 1989 to 26% in July 1991. This decline was not accompanied by an increase in the proportion of dead unionids exhibiting byssal threads. Proportions of dead unionids showing no evidence of infestation increased from 23% in September 1989 to 74% in July 1991.

There was substantial variation in intensity of infestation between sampling periods (Table 2). Total mean intensities per sampling period ranged between 79 and 7022 individuals per unionid and 3 and 37 g per unionid, whereas intensities on individual unionids ranged between 4 and 14 393 per unionid and 1 and 104 g per unionid. Mean intensities on successive sampling dates indicate that infestation declined in winters and increased in summer. Mean intensities of live and dead unionids were similar in September 1989 and higher for live than for dead unionids during May–June 1990. Maximum individual intensities of live and dead unionids were 11 550 and 14 393 unionid, respectively. In September 1989, mean weights of infesting mussels exceeded unionid mean weights by a factor of about three; in May–June 1990, infestation weights dropped to about one-third of host unionids; in September 1990, weights of mussels and unionids were about equal; and, in July 1991, weights of mussels were substantially lower than weights of unionids.

The zebra mussel population was composed of two size groups or age-classes, which declined substantially during the winter months when unionids bury in substrates (Fig. 4). The total density of infesting mussels was 7022 per unionid

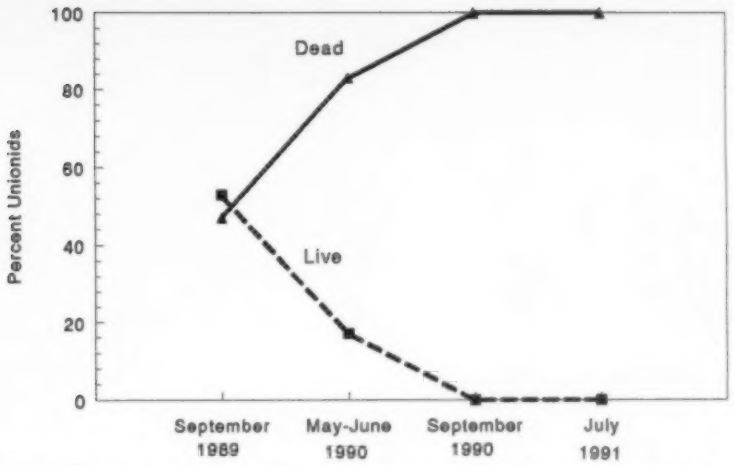

FIG. 3. Percent live and dead unionids collected at index station 9L in western Lake Erie 1989–91.

TABLE 1. Proportions (%) of live and dead unionids showing infestation (attached mussels and byssal threads only) and no infestation by zebra mussels (*Dreissena polymorpha*) at an index station (9L; Fig. 2). in western Lake Erie 1989–91.

	Sept. 18, 1989	May 20, June 26, 1990	Sept. 20, 1990	July 11 1991
Showing infestation				
Attached mussels				
Live	100	100	—	—
Dead	77	42	61	26
Byssal threads only				
Live	0	0	—	—
Dead	0	22	7	0
Total				
Live	100	100	—	—
Dead	77	64	68	26
No infestation				
Live	0	0	—	—
Dead	23	36	32	74

in September 1989, 260 per unionid in May–June 1990, 1124 per unionid in September 1990, and 79 per unionid in July 1991. Increased numbers of zebra mussels between successive sampling periods only occurred between May–June 1990 and September 1990 when small freshly spawned mussels increased densities from 248 per unionid to 1049 per unionid. In general, the mean density of smaller mussels was greater than the density of older, larger mussels, except in July 1991 when large mussels were about three times more abundant than small mussels. In September 1990 and July 1991, many of the zebra mussels, especially the larger mussels, were loosely attached and some appeared freshly dead, as evidenced by open shells and decaying tissues.

Length–frequency distributions of zebra mussels on live and dead unionids were different in September 1989 and May–June 1990 (Fig. 5). In September, live unionids were colonized by more mussels of most length classes than were dead unionids. In May–June 1990, more mussels of most length classes colonized dead rather than live unionids.

Seventeen Stations

In 1991, no live unionids were collected with the Ponar sampler at 17 historically sampled stations where live unionids had been found in the previous three decades (Table 3). Unionid diversity and abundance declined from 1961 to 1982 when five unionid species occurred at 6 of 17 stations (35%) and the mean density at all stations was $4/m^2$. A total of 23 dead unionids representing 5 unionid species (*Lampsilis siliquoidea* (15), *Ligumia nasuta* (3), *Potamilus alatus* (3), *Amblema plicata plicata* (1), and *Fusconaia flava* (1) were found.

Only four live specimens were collected with the epibenthic sled at the 17 stations in June–July 1991; three were *Amblema plicata plicata* (from stations 11D, 14D, and 2L) and one was *Fusconaia flava* (from station 14D); however, a total of 187 dead specimens representing 10 species, mostly *Lampsilis siliquoidea*, *Ligumia nasuta*, *Potamilus alatus*, and *A. p. plicata*, were collected (Table 4). The largest number

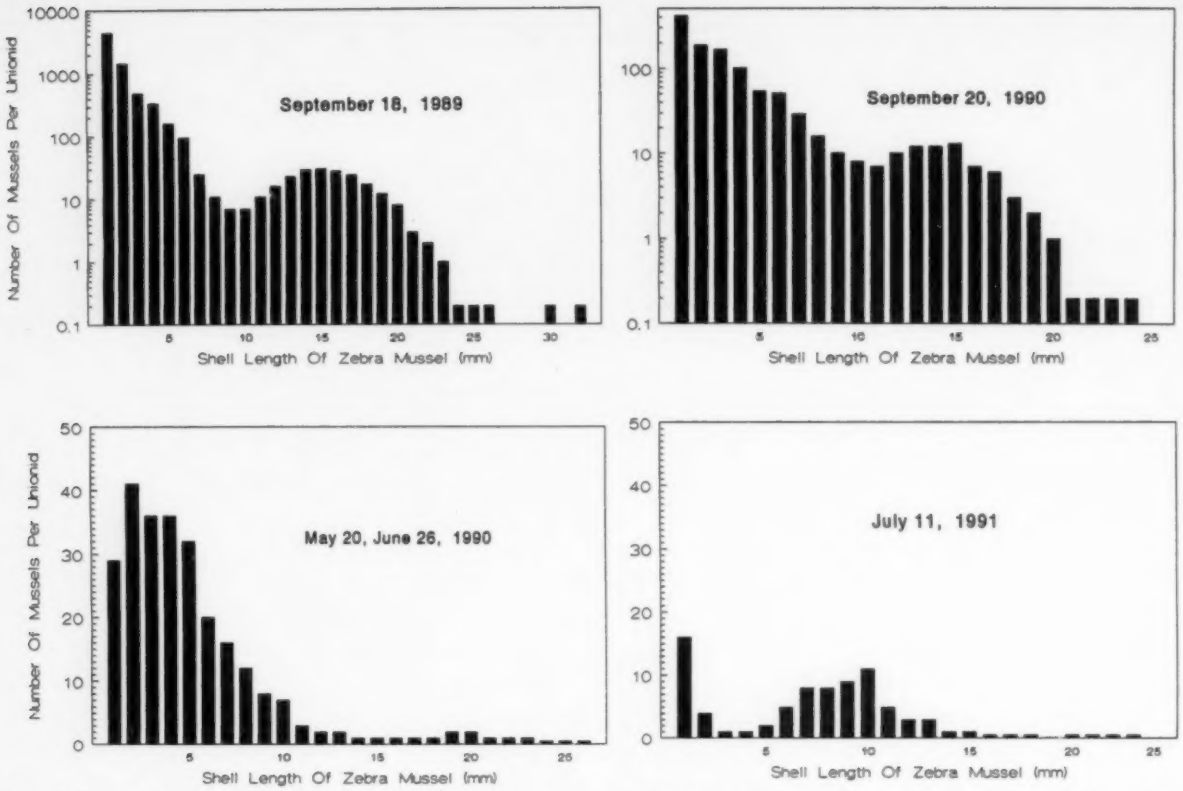
Number Of Mussels Per Unionid
September 18, 1989
Shell Length Of Zebra Mussel (mm)
September 20, 1990
Shell Length Of Zebra Mussel (mm)
May 20, June 26, 1990
Shell Length Of Zebra Mussel (mm)
July 11, 1991
Shell Length Of Zebra Mussel (mm)

FIG. 4. Length–frequency distributions of zebra mussels (*Dreissena polymorpha*) removed from unionids collected at index station 9L in western Lake Erie 1989–91.

TABLE 2. Mean (± SE) numbers and dry weights of zebra mussels (*Dreissena polymorpha*) per infested unionid, and in parentheses, numbers and mean dry weight of unionids at an index station (9L; Fig. 2) in western Lake Erie 1989–91.

	Live		Dead		Total	
	No.	Weight (g)	No.	Weight (g)	No.	Weight (g)
Sept. 18, 1989	6805±622.7 (29)	36±3.5 (14±1.7)	9120±1218.7 (3)	48±9.52 (15±2.2)	7022±584.0 (32)	37±3.3 (15±1.5)
May 20, June 26, 1990	346±95.0 (9)	9±2.1 (19±4.1)	216±98.5 (19)	5±1.7 (24±3.7)	260±73.4 (28)	7±1.3 (22±2.8)
Sept. 20, 1990			1225±218.7 (33)	13±3.4 (16±1.8)	1225±218.7 (33)	13±3.4 (16±1.8)
July 11, 1991			79±24.4 (12)	3±1.4 (17±1.7)	79±24.4 (12)	3±1.4 (17±1.7)

of dead unionids was collected at station 5L (61 specimens), located about in the middle of the sampled area.

Discussion

Present Study

Our study reveals that live Unionidae were nearly extirpated in a large portion of western Lake Erie between 1989 and 1991. Live unionids were present at an index station in 1989 but disappeared by 1990, and no live unionids were found in 1991 at 17 quantitatively sampled stations, where unionids had been present for decades. In addition, qualitative sampling revealed that only 4 of 191 total specimens were live, and all of these were infested by zebra mussels. Dramatic exponential increases of zebra mussel densities occurred in western Lake Erie in 1989 (reviewed in Nalepa and Schloesser 1993). These increased densities of mussels coincided with rapid and massive infestation of unionids and their subsequent decline in western Lake Erie. We believe that the invasion of zebra mussels into western

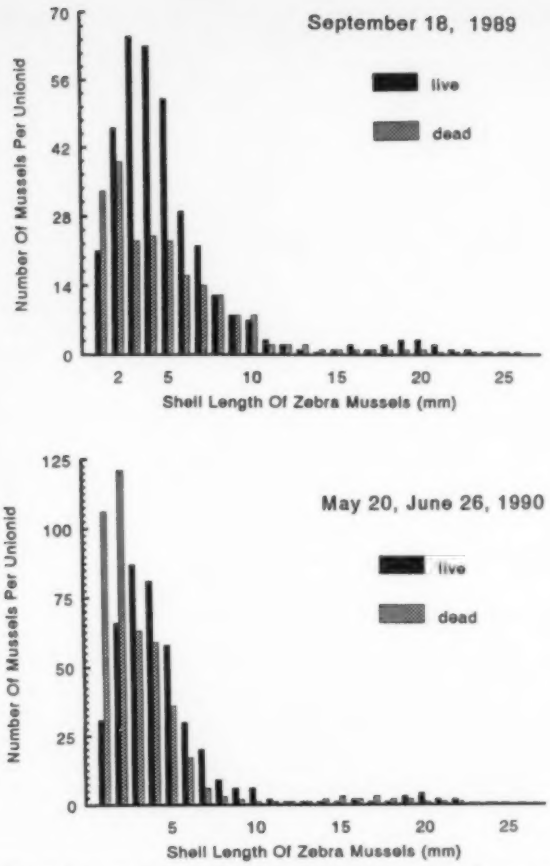

FIG. 5. Length–frequency distributions of zebra mussels (*Dreissena polymorpha*) removed from live and dead unionids collected at index station 9L in western Lake Erie September 1989 and May–June 1990.

Lake Erie in 1986 (Griffiths et al. 1991), plus the dramatic increase in mussel densities and massive infestation of unionids in the summer of 1989, indicate that zebra mussels caused near extinction of unionids in a large portion of western Lake Erie by 1990.

Infestation intensities, length–frequency distributions of infesting zebra mussels, and proportions of unionids showing evidence of infestation indicate that unionid mortality due to zebra mussels primarily occurred shortly after the present study was initiated. In fall 1989, infestation intensities and length–frequency distributions of zebra mussels on live and dead unionids were similar, indicating that infested live and dead unionids collected in fall 1989 were exposed to the same exponential population growth of mussels that occurred in the summer of 1989 (Nalepa and Schloesser 1993). In fall 1989, most dead, and all live unionids, showed no evidence of being buried in soft sediments at the index station, indicating that they had maintained themselves at the sediment–water interface during the summer of 1989. However, a few dead unionids appeared to have been buried in sediments for a short period (as evidenced by odor, decaying tissues, and the presence of black subsurface sediments in mussel and unionid shells); such mortality is attributed to the inability of unionids to maintain themselves at the water–sediment interface because of the three-fold increase in weight of infesting mussels and subsequent smothering in the sediment. Dead unionids that showed no infestation (23%) were mostly old, decalcified specimens that appeared to have been buried and not exposed to zebra mussel colonization in 1989. By spring 1990, length– frequency distributions of mussels on dead unionids were substantially different than those for mussels on live unionids, and 22% of dead unionids showed evidence (i.e., byssal threads attached) of having been infested. Most of the small number of zebra mussels that were attached to unionids in spring 1990 were dead, black, smelled of decaying flesh, and appeared to have been buried in substrates when sampled. These freshly dead zebra mussels probably died from suffocation during winter months when unionids typically burrow into the substrate (Morton 1993). In addition, freshly dead unionids were found in spring 1990 and fall 1990. No freshly dead unionids were found in summer 1991.

TABLE 3. Percent composition of live unionid species collected with a quantitative sampler at 17 historically sampled stations (Fig. 2) in western Lake Erie 1961, 1972, and 1982 (Nalepa et al. 1991), and 1991 (present study).

	1961	1972	1982	1991
Lampsilis siliquoidea	33	63	40	0
Ligumia nasuta	33	8	30	0
Pyganodon grandis	17	8	10	0
Potamilus ohiensis	6	8	0	0
Elliptio dilitata	3	0	0	0
Obliquaria reflexa	3	0	0	0
Leptodea fragilis	3	0	0	0
Potamilis alatus	3	4	10	0
Lampsilis cardium	0	8	0	0
Amblema plicata plicata	0	0	10	0
No. of Species	8	6	5	0
Stations with unionids (%)	94	65	35	0
Mean No./m^2	10	6	4	0

Increasing proportions of dead unionids and unionids showing evidence of infestation would be expected throughout the period of study if the zebra mussel infestation was the primary cause of mortality. However, observations of byssal threads attached to unionid shells buried in sediments of western Lake Erie and artificially suspended in the water column indicate that visible evidence of zebra mussel infestation only remains on unionids for 3–4 mo after unionids die and are buried in soft sediments (D.W. Schloesser, personal observation). Thus, the use of byssal threads as an indicator of unionid infestation and resulting mortality is possible for only a short period of time after unionids become buried in soft sediments.

Historical Unionid Studies

The near total absence of live Unionidae in 1990 and 1991, and the speed at which the decline occurred between 1989 and 1991 is historically unprecedented in the offshore waters of western Lake Erie. Nalepa et al. (1991) documented the long-term decline of Unionidae in the western basin using available data collected with Ponar-type samplers from several previous studies. Nalepa's study showed that densities of unionids declined from 10/m^2 in 1961 to 6/m^2 in 1972 to 4/m^2 in 1982; the proportion of stations

TABLE 4. Number of dead unionid species collected in an epibenthic sled (5 minute tows) at 17 stations (Fig. 2) in western Lake Erie June 17 and July 11, 1991.

Species	1L	2L	3L	4L	5L	6L	7L	8L	9L	10L	4D	7D	11D	14D	4R	5R	5M	Total
Lampsilis siliquoidea	1	18	4	3	40	4	2	2		1				14	1	23		113
Amblema plicata plicata		3	2									1			1	2		9
Potamilus alatus		3			6		1	2										12
Elliptio complanata		2			2											2		6
Pyganodon grandis			2		4													6
Ligumia nasuta					8	2	1	2						17		5		35
Ligumia recta					1									1				2
Obliqueria reflexa																1		1
Quadrula pustulosa																1		1
Fusconaia flava																2		2
Total	1	26	8	3	61	6	4	6		1		1		32	2	36		187

with live unionids declined from 94% in 1961 to 65% in 1972, to 35% in 1982, while the number of species declined from 8 in 1961, to 6 in 1972, to 5 in 1982. Although the reason for the long-term decline is uncertain, Nalepa et al. (1991) believed it to be related to reduced water and substrate quality. Between 1982 and the present study, unionid densities at the index station declined from 4 to 0/m^2, the percentage of stations with unionids declined from 35 to 0%, and the number of unionid species declined from 5 to 0. The near total absence of live unionids at the 17 stations sampled by the Ponar sampler is confirmed by results from epibenthic sled samples. Species composition of dead unionids in sled samples was similar to the composition of live unionids found in earlier studies (Nalepa et al. 1991).

Although declines of unionid abundance have been documented throughout western Lake Erie for every 10-yr period since 1961, the rapid decline of Unionidae at the index station between fall 1989 and spring 1990 and the total absence of live unionids in fall 1990 and summer 1991 indicate that substantial changes occurred which affected unionid survival over a very brief time. Rapid declines of unionids in other water bodies have been attributed to diseases, parasites, short-term fluctuations in water quality, and unknown causes (reviewed by Neves 1987). Examination of unionids in fall 1989 revealed no morphological evidence of unionid disease or parasites. Historically, western Lake Erie has been subject to catastrophic, short-term declines in water quality, such as low oxygen levels (Britt 1955; Carr and Hiltunen 1965; Wood 1973). However, observations while sampling in the present study indicate good habitat conditions, as evidenced by the presence of other live benthic organisms (i.e., amphipods, isopods, oligochaetes, chironomids, etc.) throughout the sampled area. In addition, benthic quality surveys of western Lake Erie indicate that habitat conditions improved dramatically between 1979 and 1991 (Farara and Burt 1993) and were good in 1993 (as evidenced by the presence of *Hexagenia* spp. nymphs; D.W. Schloesser, unpublished data). These surveys indicate that improved habitat conditions may have allowed the historically declining unionid populations to increase in abundance in western Lake Erie. However, if unionid recovery in western Lake Erie is to occur, it will probably take much longer after the severe mortality caused by the zebra mussel.

Other Zebra Mussel – Unionid Interaction Studies

The dramatic decline of unionids as documented in the present study is similar to that in Lake St. Clair located immediately upstream of western Lake Erie (Fig. 2; Nalepa 1994; Gillis and Mackie 1994). Potential negative effects of zebra mussels on unionids in the Great Lakes were first noted in Lake St. Clair in 1988 by Hebert et al. (1989), who found a maximum of 35 mussels on one unionid and suggested that zebra mussels may be an interference competitor of native unionids. After zebra mussels spawned in Lake St. Clair in 1989, Hebert et al. (1991) noted a maximum infestation intensity exceeding 10 000 mussels per unionid but provided no individual data. Hebert et al. (1991) determined that infested unionids had 50% lower levels of lipids in body tissues than noninfested unionids and suggested that the long-term survival of unionids in Lake St. Clair may be threatened. Additional studies by Mackie (1991, 1993) in Lake St. Clair indicated a maximum infestation intensity of 625 and a mean intensity of 210 mussels per unionid. Nalepa (1994) surveyed Lake St. Clair and found that in areas of high zebra mussel infestation (>55 per unionid) unionids disappeared in a large portion of the lake between 1990 and 1992. Nalepa found some unionid populations in northwestern Lake St. Clair where infestation intensities were below 3 per unionid.

Near extirpation of unionids in Lake Erie occurred about 1 yr (1990) earlier at the index station in the present study than in southern Lake St. Clair (1991), even though zebra mussels invaded both areas in 1986 (Gillis and Mackie 1994; Griffiths et al. 1991). In general, mean intensities in western Lake Erie are about 7- to 10-fold higher than in Lake St. Clair (Hunter and Bailey 1992; Leach 1993). In 1989, Schloesser and Kovalak (1991) determined that unionids in nearshore waters of western Lake Erie were infested by mussels at a maximum intensity of 10 732 and a mean intensity of 6777 per unionid and suggested that the survival of unionids with these intensities was unlikely. In the present study, maximum infestation intensities of live (11 550) and dead (14 393) unionids exceed those reported earlier.

Higher infestations in western Lake Erie than Lake St. Clair are attributed to differences in substrate densities of zebra mussels. Maximum densities in western Lake Erie have been reported to exceed 342 000/m^2, whereas in Lake St. Clair maximum densities have not exceeded 19 000/m^2 (Leach 1993; Hunter and Bailey 1992). As shown by Lewandowski (1976), there is a direct relationship between unionid infestation rates and relatively low environmental densities of zebra mussels. At low environmental densities in Europe, there are few documented effects of infestation on unionids (Lewandowski 1976; Schloesser and Kovalak 1991). However, high infestation

intensity did occur in Lake Balaton, Hungary, in the 1930s and caused high unionid mortality (Sebestyen 1938; reviewed by Schloesser and Kovalak 1991).

Mechanisms of Unionid Mortality

At present, the causal mechanism(s) of increased mortality of unionids as a result of zebra mussel infestation is unknown. This is partly because declines have only recently been attributed to mussel infestations, and because it is difficult to separate interactions between habitat characteristics and infestation impacts. There are several hypothetical causal mechanisms, which may not be the same for all habitat conditions (Schloesser and Kovalak 1991; Mackie 1991).

— impair normal locomotion and burrowing activities
— prevent valve closure, thus exposing the unionid to predation, parasitism
— prevent valve opening, thus stopping respiration
— smother siphons, thus stopping metabolic activities
— reduce available food for the unionid
— cause shell deformities, thus preventing normal growth
— generate metabolic wastes, thus causing toxic effects
— add weight to the unionid shell, thus causing unionid to sink into soft sediments and be smothered

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However, even at relatively low infestation rates and low environmental densities of zebra mussels, unionids have shown physiological effects (e.g., reduced lipid content, Hebert et al. 1991; reduced glycogen content, Haag et al. 1993) and complete mortality (Gillis and Mackie 1994). A reduction in metabolic reserves of infested unionids has been observed in controlled studies in western Lake Erie and in natural populations in Lake St. Clair (Hebert et al. 1991; Haag et al. 1993).

We attribute unionid mortality to reduced metabolic reserves, as evidenced by high mortality and poor condition of unionids collected in spring. Many dead unionids collected in spring 1990 contained decaying flesh and appeared to have been buried in the soft sediments. Reduced metabolic reserves of unionids caused by infesting mussels in the summer 1989 combined with increased weight of infesting mussels prevented unionids from surviving winter and (or) prevented burrowing unionids from reaching the substrate surface in 1990 when water temperatures increased.

We have documented dramatic reductions in unionid populations in western Lake Erie. The rapid, large invasion of the study area by zebra mussels corresponded with a near 100% mortality of unionids. To date, all studies support predictions (French 1990; Hebert et al. 1991; Schloesser and Kovalak 1991) that high densities of zebra mussels will result in high infestation intensities and that these would cause mortality of unionids in the Great Lakes, and possibly throughout much of North America.

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References

BRITT, N.W. 1955. Stratification in western Lake Erie in summer of 1953; effects on the *Hexagenia* (Ephemeroptera) population. Ecology 36: 239–244.

CARR, J.F., AND J.K. HILTUNEN. 1965. Changes in the bottom fauna of western Lake Erie from 1930 to 1961. Limnol. Oceanogr. 10(4): 551–569.

CLARKE, A.H. 1981. The freshwater molluscs of Canada. National Museum of Natural Sciences, Ottawa, Canada.

FARARA, D.G., AND A.J. BURT. 1993. Environmental assessment of western Lake Erie sediments and benthic communities—1991. Report: Ontario Ministry of Environment and Energy, Water Resources Branch, Great Lakes Section, Toronto, Ont.

FRENCH, J.R.P., III. 1990. The exotic zebra mussel—a new threat to endangered freshwater mussels. Endangered Species Tech. Bull. XV(11), U.S. Fish and Wildlife Service, Washington, D.C. p. 3–4.

GANNON, J.E., D.W. SCHLOESSER, AND E. KAFCAS. 1990. Effects of the exotic zebra mussel on indigeneous species in the Great Lakes. Abstracts of the American Society of Limnology and Oceanography, Williamsburg, Va.

GARTON, D.W., AND W.R. HAAG. 1991. Heterozygosity, shell length and metabolism in the European mussel, *Dreissena polymorpha*, from a recently established population in Lake Erie. Comp. Biochem. Physiol. 99A(1/2): 45–48.

GILLIS, P.L., AND G.L. MACKIE. 1994. Impact of the zebra mussel, *Dreissena polymorpha*, on populations of Unionidae (Bivalvia) in Lake St. Clair. Can. J. Zool. 72: 1260–1271.

GRIFFITHS, R.W., W.P. KOVALAK, AND D.W. SCHLOESSER. 1989. The zebra mussel, *Dreissena polymorpha* (Pallas, 1771), in North America: impact on raw water users, p. 11–27. *In* Proceedings of EPRI Service Water System Reliability Improvement Seminar. Nov. 6–8, 1989, Charlotte, N.C. Electric Power Research Institute, Palo Alto, Calif.

GRIFFITHS, R.W., D.W. SCHLOESSER, J.H. LEACH, AND W.P. KOVALAK. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes. Can. J. Fish. Aquat. Sci. 48: 1381–1388.

HAAG, W.R., D.J. BERG, D.W. GARTON, AND J.L. FARRIS. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Can. J. Fish. Aquat. Sci. 50: 13–19.

HEBERT, P.D., B.W. MUNCASTER, AND G.L. MACKIE. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. 46: 1587–1591.

HEBERT, P.D., C.C. WILSON, M.H. MURDOCH, AND R. LAZAR. 1991. Demography and ecological impacts of the invading mollusc *Dreissena polymorpha*. Can. J. Zool. 69: 405–409.

HUNTER, R.D., AND J.F. BAILEY. 1992. *Dreissena polymorpha* (zebra mussel): colonization of soft substrata and some effects on unionid bivalves. Nautilus, 106(2): 60–67.

LEACH, J.H. 1993. Impacts of the zebra mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs in western Lake Erie, p. 381–397. *In* T.F. Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis CRC Press, Inc., Boca Raton, Fla.

LEWANDOWSKI, K. 1976. Unionidae as a substratum for *Dreissena polymorpha* Pall. Pol. Arch. Hydrobiol. 23: 409–420.

MACKIE, G.L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia, 219: 251–268.

MACKIE, G.L. 1993. Biology of the zebra mussel (*Dreissena polymorpha*) and observations of mussel colonization on unionid bivalves in Lake St. Clair of the Great lakes, p. 153–166. *In* T.F. Nalepa and D.W. Schloesser [ed.]. Zebra mussels: biology, impacts, and control. CRC Press Inc., Boca Raton, Fla.

MARGOLIS, L., G.W. ESCH, J.C. HOLMES, A.M. KURIS, AND G.A. SCHAD. 1982. The use of ecological terms in parasitology. J. Parasitol. 68: 131–133.

MILLER, A.C., R. WHITING, AND D.B. WILCOX. 1989. An evaluation of a skimmer dredge for collecting freshwater mussels. J. Freshwater Ecol. 5(2): 151–154.

MORTON, B. 1993. The anatomy of *Dreissena polymorpha* and the evolution and success of the heteromyarian form in the Dreissenoidea. p. 185–216. *In* T.F. Nalepa AND D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. CRC Press, Inc., Boca Raton, Fla. 810 pp.

NALEPA, T.F. 1994. Decline of native unionid bivalves in Lake St. Clair after infestation by the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 51: 2227–2233.

NALEPA, T.F., B.A. MANNY, J.C. ROTH, S.C. MOZLEY, AND D.W. SCHLOESSER. 1991. Long-term decline in freshwater mussels (Bivalvia: Unionidae) of the western basin of Lake Erie. J. Gt. Lakes Res. 17(2): 214–219.

NALEPA, T.F., AND D.W. SCHLOESSER. [ed.] 1993. Zebra Mussels: biology, impacts, and control. Lewis CRC Press, Inc., Boca Raton, Fla.

NEARY, B.P., AND J.H. LEACH. 1992. Mapping the potential spread of the zebra mussel (*Dreissena polymorpha*) in Ontario. Can. J. Fish. Aquat. Sci. 49: 406–415.

NEVES, R.J. [ed.] 1987. Proceedings of the workshop on die-offs of freshwater mussels in the United States. U.S. Fish. and Wildlife Service and Upper Mississippi River Conservation Committee, Rock Island, Ill.

SBLENDORIO, R.P., J.C. MALINCHOCK, AND R. CLAUDI. 1991. Controlling zebra mussel infestations at hydroelectric plants. Hydro Rev. X(4): 42–50.

SCHLOESSER, D.W., AND W. KOVALAK. 1991. Infestation of unionids by *Dreissena polymorpha* in a power plant canal in Lake Erie. J. Shellfish Res. 10(2): 355–359.

SCHLOESSER, D.W., T.F. NALEPA, AND K. KAFCAS. 1990. Exotic zebra mussels may affect indigeneous species in North America. Res. Inf. Bull. 90-40. U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C.

SEBESTYEN. O. 1938. Colonization of two new fauna-elements of Ponto-origin (*Dreissena polymorpha* Pall. and *Corophium curvispinum* G.O. Sars froma devium Wundsch) in Lake Balaton. Verh. Int. Ver. Theor. Angew. Limnol. 8: 169–182.

STRAYER, D.L. 1991. Projected distribution of the zebra mussel, *Dreissena polymorpha* in North America. Can. J. Fish. Aquat. Sci. 48: 1389–1395.

TURGEON, D.D., A.E. BOGEN, E.V. COAN, W.K. EMERSON, W.G. LYONS, W.L. PRATT, C.F.E. ROPER, A. SCHELTEMA, F.G. THOMPSON, AND J.D. WILLIAMS. 1988. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks. Am. Fish. Soc. Spec. Publ. 16.

WILLIAMS, J.D., M.L. WARREN, K.S. CUMMINGS, J.L. HARRIS, AND R.J. NEVES. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries, 18(9): 6–22.

WOOD, K.G. 1973. Decline of *Hexagenia* (Ephermeroptera) nymphs in western Lake Erie. p. 26–32. *In* W.L. Peters and J.G. Peters [ed.] Proceedings of the 1st International Conference on Ephemeroptera, 17–20 Aug. 1970, Florida A & M University, Tallahassee, Fla.

The 25th Anniversary of the Experimental Lakes Area and the History of Lake 227

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Johnson and Vallentyne (1971) traced the original impetus for the Experimental Lakes Area (ELA), and whole-ecosystem experimentation, to a recommendation in a 1965 report of the International Joint Commission (IJC) concerning pollution in the lower Great Lakes. The recommendation called for "...short-term studies to assess the sources and nature of the pollutants and longer-term research and monitoring programmes to assure the best solutions" (Johnson and Vallentyne 1971, p. 123). As a result of the recommendation, the Fisheries Research Board "...was assigned responsibility for studies involving biological productivity and for a greater understanding of eutrophication—the important problem of nutrient pollution" (Johnson and Vallentyne 1971, p. 123). The resulting program included "...experimental eutrophication studies on small lakes to determine quantitative relations, overall ecological effects, persistence of nutrient pollutants, and remedial measures" (Johnson and Vallentyne 1971, p. 123). And so the ELA in northwestern Ontario was born.

Initial surveys to select an area of suitable lakes began by helicopter in 1967 (Cleugh and Hauser 1971). Surveys were continued in 1968 from a temporary camp on Lake 132. A research station was constructed at the present site, Lake 239 (Rawson Lake), in 1969–1970.

Experimental nutrient additions to Lake 227 were initiated in June 1969. This was the first of many whole-ecosystem experiments carried out at the ELA to resolve pressing issues in lake and fisheries management.

The ELA was only part of a broader Canadian response to the IJC recommendation, but it filled a critical void between uncontrolled, observational studies on natural and polluted lakes and closely controlled, but necessarily artificial, laboratory experiments. Johnson and Vallentyne (1971) did not envision whole-ecosystem experimentation replacing either of these other research approaches, but they saw it as a necessary complement to what is a continuum of investigative scales ranging in size from test tubes to the largest lakes in the world. They saw the experimental manipulation of whole ecosystems as a means of testing ecological effects and responses with an increasing degree of reliability and applicability to "real-world" systems. They also argued that, if aquatic ecology was to become an "exact" science, it must be able to experimentally test hypotheses at an appropriate scale. For many issues confronting aquatic science today, the ecosystem is the appropriate scale. It is at this scale that the ELA continues to focus its attention, and has made its most substantive contributions.

Nutrient additions were still being made to Lake 227 in June 1994, marking the 25th consecutive year of artificial enrichment. Perhaps the anniversary of this experiment most appropriately serves as a temporal marker for the overall ELA program. Twenty-five years is but an instant in the history of a lake, but it is a significant milestone in the history of a scientific program (especially for a science as young as ecology, the intellectual origins of which are not much older than a century) or, certainly, of a whole-ecosystem experiment. The ELA did not invent whole-ecosystem experiments; Johnson and Vallentyne (1971) clearly recognized the contributions and insights of others in this regard. However, it is rare to continue active experimentation on a natural system for such a long time.

To commemorate the 25th anniversary of the ELA program, three series of papers are being published in the *Canadian Journal of Fisheries and Aquatic Sciences*. The first series, comprising the following eight papers, is devoted to research results from Lake 227, thereby also celebrating the 25th anniversary of that experiment. The second series of seven papers will follow later this year and will be devoted to natural processes in lakes. The third series is scheduled to appear in 1995 and will treat recent research on contaminant issues.

What possible reason can there be for continuing an experiment for 25 yr? The easy answer is that it has not been one experiment, but three. Although artificial phosphorus (P) loading has been essentially constant for 25 yr, nitrogen (N) has been added at three different (progressively reduced) rates over that time (Findlay et al. 1994). The better, if less obvious, answer is that some questions can only be answered after study over a significant length of time. Even if the experiment is considered in its simplest context, i.e., constant P loading over time, strong justification exists for its long duration. In fact, results suggest that 25 yr may actually be the minimum time required to appreciate fully the effects of a significant perturbation on an aquatic ecosystem. Aquatic organisms have generation times ranging from hours for microbes to years for fish. Consequently, the time required for an aquatic ecosystem to approach a new "steady state" after perturbation can easily extend to decades for the populations of longest-lived organisms; perhaps even more time may be required if significant feedbacks exist among long-lived organisms and those with short generation times. This fact may be as disconcerting to funding agencies as it is to ecosystem scientists who also have limited career life expectancies. Whole-ecosystem experiments of this duration will probably remain a rarity, but are extremely important for the assessment of the limitations of shorter experimental manipulations. The papers in this issue present many examples of delayed responses: at higher trophic levels (Hann et al. 1994); to new quasi-steady states (Findlay et al. 1994; Wolfe et al. 1994); adjustments over time illustrating feedbacks among trophic levels (Leavitt et al. 1994); long-term changes that challenged initial interpretations

(Hendzel et al. 1994); and changing taxonomic nomenclature (Kling et al. 1994).

The Lake 227 experiment demonstrated that certain questions can only be addressed after the passage of considerable time. Paleolimnology is increasingly accepted and applied as an investigative method for demonstrating past change and perturbation in lakes. Many anthropogenic pollutants are generated at slow rates or are not identified as pollutants until long after their release to the environment. Paleolimnology is often the only means of assessing the environmental history and effects of such pollutants, or slowly occurring natural change. However, paleolimnology is most often applied to lakes that have not been studied over the duration of anthropogenic disturbance. Consequently, independent verification of this method of historical reconstruction is rare. It is even rarer when the experimental perturbation is as precisely known and the monitoring studies are as comprehensive as in Lake 227.

Paleolimnology cannot be done without good stratigraphy and independent methods to establish a relative or, preferably, an absolute chronostratigraphy. Fortunately, Lake 227 produces at its greatest depths laminated sediments that are recognizable to the unaided eye; the lake has been regularly producing these laminae for hundreds of years. Wolfe et al. (1994) used a combination of radioisotopic and biostratigraphic techniques to establish that most of these laminations are indeed true varves (i.e., paired couplets of light and dark sediments repetitively produced by annual cycles of sedimentation). Experimental nutrient addition complicated the lamination story because additional algae-derived laminae were produced in some years when the lake was highly eutrophic (Wolfe et al. 1994); nevertheless, age assignment is as precise as achievable. Consequently, these varves provide an independent, absolute time scale for a year-by-year analysis of sediment constituents, which can be compared with the known record over the past 25 yr. Hence, the fidelity and resolution of the paleolimnological method can be defined. Paleolimnology also provided previously unknown and unstudied information about the effects of eutrophication on Lake 227 (e.g., Zeeb et al. 1994; Hann et al. 1994).

The Lake 227 experiment was originally designed to answer a single question that vexed algal ecologists and hampered lake restoration proposals in the 1960s (Schindler 1971): Is artificial carbon (C) enrichment essential to the eutrophication process? Lake 227, isolated and pristine, was ideally suited to answer the question because of its naturally low dissolved inorganic C content. Nitrogen and P were added to the lake at areal loading rates comparable to those then occurring in the lower Great Lakes in the absence of artificial C enrichment. The response of the algal community was dramatic and immediate (Findlay et al. 1994). It was demonstrated conclusively that the atmosphere was an inexhaustible source of carbon dioxide to Lake 227, which allowed algal populations to utilize all the N and P added (Schindler et al. 1972; Emerson et al. 1973).

Having shown that the atmosphere could supply C without limit to sustain high algal biomass, it was decided to reduce N loading in 1975 to evaluate the role of N in producing and sustaining high algal biomass. Could reducing N loading alone cause a reduction in high algal biomass in Lake 227, which had been dominated by chlorophytes and filamentous cyanoprokaryotes (blue–green algae) since artificial fertilization began (Findlay et al. 1994)? Cyanoprokaryotes often dominate algal biomass in highly eutrophic lakes; various hypotheses exist to explain this dominance (Findlay et al. 1994). One of these hypotheses could be tested by lowering the N loading rate relative to P.

A low N:P loading ratio was expected to provide a competitive advantage to heterocystous, N_2-fixing cyanoprokaryotes, which can draw on atmospheric N to meet their N requirement for growth and, thereby, fully use available P (Flett et al. 1980). Reduced artificial N loading did induce the development of N_2-fixing cyanoprokaryotes of the genus *Anabaena* and, subsequently, *Aphanizomenon schindleri* Kling, Findlay, and Komárek (Kling et al. 1994). Algal biomasses in the years after the 1975 reduction in N loading were equal to, or greater than, before reduction (Findlay et al. 1994). This result clearly demonstrated that reduced N loading alone in most lakes would not be an efficacious strategy to reduce undesirable algal growth because an infinite atmospheric source would remain available to N_2-fixing cyanoprokaryotes.

By the mid 1980s, further changes in the algal community in Lake 227 appeared to question the generality of the initial results of the 1975 reduction in N loading. The historic maximum algal bloom in 1982, which was dominated by *A. schindleri*, was followed by several years of lower biomass in which *A. schindleri* was a relatively minor component of the phytoplankton (Findlay et al. 1994). Again, several hypotheses (e.g., onset of trace-metal limitation; insufficient excess P to favor N_2-fixing cyanoprokaryotes; increased N loading from littoral sediments) were considered and evaluated in limnocorral experiments (Hendzel et al. 1994). The most likely explanation again involved the N:P loading ratio to the phytoplankton and recognition of the increased importance of internal N loading, which had substantially increased after many years of eutrophication. To test this hypothesis, artificial N loading to Lake 227 was terminated in 1990, whereas artificial P loading continued unchanged. The expectation (and subsequent reality) was that N_2-fixing cyanoprokaryotes would again dominate in a predictable manner after the elimination of artificial N loading (Findlay et al. 1994). In fact, in the summer of 1994, a spectacular bloom, the largest since 1982 and essentially a monoculture of *A. schindleri*, occurred in Lake 227. This was the fifth consecutive year of *A. schindleri* dominance after artificial N loading ceased.

The P content of the lake had quickly established a new steady state after artificial loading began in 1969 (Schindler et al. 1987), but a steady state for N was not established until much later. N_2-fixation eventually brought the N content of the lake to a new steady state (Hendzel et al. 1994; Findlay et al. 1994) by increasing the internal store of N. In turn, the internal loading of N increased, the N deficit to be met by fixation decreased, and the prominence of N_2-fixing *Aphanizomenon* decreased. Because the steady state internal store of N is set by the internal store of P, which has remained constant, further increases in internal N loading are not expected and summer N_2-fixation by *A. schindleri* will be necessary to balance the N demand in Lake 227 until the P loading rate is altered (Findlay et al. 1994). The different dynamics of N and P cycling could not have been appreciated without extending the experiment over two decades. This illustrates the uncertainty of interpreting and applying results from short-term experiments to whole ecosystems, which may have quite long response times.

The paleolimnological results illustrate a remarkable fidelity to the known history of Lake 227 (Leavitt et al. 1994). The most recent cores analyzed were taken in 1990, so the effects of elimination of artificial N loading could not be studied. However, fossil pigments, diatoms, chrysophytes, and Cladocera, which leave identifiable remains, all clearly discriminated the pre- from the post-fertilization period and the two different experimental N:P loading regimes. Bulk accumulation rates and elemental composition in two cores recovered from the deepest part of the lake were less sensitive than might be expected to an order of magnitude increase in P loading, algal photosynthesis, and biomass (Wolfe et al. 1994; Leavitt and Findlay 1994). This may have been a reflection of naturally high rates of organic sedimentation fed allochthonously from the surrounding boreal forest and/or altered depositional patterns within the lake (i.e., much increased shallow-water sedimentation), neither of which was studied. Wolfe et al. (1994) demonstrated that eutrophication altered the regular cycle of sedimentation that produced true varves for >400 yr (the length of time available in their core). In particular, summer sedimentation became more complex as various species of phytoplankton exhibited substantial blooms within the open-water season, and potentially added laminae to what were annual couplets. Simply counting laminae, rather than confirmed annual cycles, could lead to errors in age assignments as large as 6 yr for the postfertilization period (Wolfe et al. 1994). Such errors, together with possible differences in sedimentation and preservation among taxa and years, can only degrade the correlation between annual estimates of taxa production and deposition. Despite these unavoidable problems, remarkably good correlation existed between mean annual standing crops of many taxa (from historical observations) and annual abundance in their contemporaneous varve (Leavitt and Findlay 1994; Leavitt et al. 1994). Also, years of unusual abundances of some taxa were readily identified within the sediment record (Wolfe et al. 1994), further substantiating the translation of potentially ephemeral information from extant populations growing in the lake to secured and stored information in the sediments.

A rather unique contribution of the Lake 227 experiment to verification of the paleolimnologic method is the assessment of the reliability of potential contributors to the fossil record (Leavitt et al. 1994). Dinoflagellate carotenoids and nonchitinous zooplankton were unreliable, whereas chitinous Cladocera, algal microfossils, and several pigments were highly reliable. It would be overly simplistic to assume that such assessments necessarily apply to all sediment records, but at least they provide validated evidence. Perhaps more significant to the evolving science of paleolimnology is that the Lake 227 record can serve as a documented stratigraphic sequence against which other sequences thought to be determined by eutrophication can be compared. Individual sequences will differ in their particulars, but certain general responses will be common. This may not be significant in North America and Europe, which have a rich tradition of limnological observation and many lakes available for comparison along a continuum of cultural perturbation. However, as the paleolimnologic method is applied to other parts of the globe less rich in limnological experience or opportunity for comparative analysis, validation of the method against known "standard" situations will become necessary if it is to become useful as a tool for lake management. In this regard, evidence existed of prior eutrophying events in the Lake 227 history; one period corresponded with evidence of a large forest fire (Leavitt et al. 1994). However, none of these earlier events was comparable in effect to experimental eutrophication (Leavitt and Findlay 1994).

The paleolimnologic studies have enriched our understanding of the response of Lake 227 to eutrophication. The choice of ecosystem response variables and frequency of observation is always selective, given limited budgets and human talents. The pelagic community of Lake 227 was initially chosen for detailed monitoring, primarily during the open-water season; changes in the littoral and benthic communities were neglected. Accumulating sediments, however, do not have such biases, so that diatoms and chitinous Cladocera growing in the littoral zone contribute to the deep-water sediments and become part of the ecological record available to paleolimnology (Zeeb et al. 1994; Hann et al. 1994). In Lake 227, all we know about the responses of the littoral community to artificial enrichment is what we can interpret from the sediment record. The littoral diatoms apparently were just as sensitive as the pelagic community with regard to species successions after enrichment, and they also responded to the different N:P loading ratios (Zeeb et al. 1994). The species composition of the littoral chydorid community was, likewise, affected by enrichment and, surprisingly, by the nutrient-loading ratios. However, it does not appear as if the numerical abundance of the littoral chydorid community responded to enrichment. In contrast, good correspondence existed between pelagic *Bosmina* abundance and *Bosmina* remains accumulating in contemporaneous varves. Both lines of evidence confirmed a decline in *Bosmina* abundance after enrichment, but morphological analysis of *Bosmina* body parts and community size-structure analysis indicated that predation played a role in this decline (Hann et al. 1994).

What is left for the Lake 227 experiment and the ELA to contribute to aquatic science as they continue beyond their 25th year? In 1969, Lake 227 was the only whole-lake experiment and eutrophication was the only environmental issue being considered at the ELA. Today, a variety of whole-lake studies are in progress: (1) three acidification experiments involving lakes in various stages of recovery; (2) three biomanipulation experiments; (3) a cadmium-addition experiment involving lake recovery; and (4) a study of the effects of wetland flooding on littoral-zone productivity, methylmercury production, and greenhouse-gas generation. The experimental-lake approach to examining aquatic environmental issues has proven itself and has been extended into whole-ecosystem experimentation in wetland and upland ecosystems. The most satisfying recognition of the program was the awarding of the Stockholm Water Prize to Professor D.W. Schindler, the ELA's first scientific director, for his research at the ELA (Hamilton 1991). Most encouraging is the continued strong interest and widening participation in research at the ELA. In 1994, the department of Fisheries and Oceans and Environment Canada continue to operate their programs of research and monitoring. In addition, numerous scientists and graduate students from universities in four Canadian provinces and three American states are actively pursuing their research at the ELA. Lake 227 remains central to the research effort. In 1993, northern pike were introduced into Lake 227, which had previously

lacked a piscivore, to examine top-down effects on the food web and algal biomass. In 1992, Lake 227 was included in a research program initiated to examine the effect of eutrophication on concentrations of organochlorines in water and biota. The ELA and Lake 227 will continue to enrich aquatic science and the scientific basis of lake management for the foreseeable future.

References

CLEUGH, T.R., AND B.W. HAUSER. 1971. Results of the initial survey of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 129–137.

EMERSON, S., W. BROECKER, AND D.W. SCHINDLER. 1973. Gas-exchange rates in a small lake as determined by the radon method. J. Fish. Res. Board Can. 30: 1475–1484.

FINDLAY, D.L., R.E. HECKY, L.L. HENDZEL, M.P. STAINTON, AND G.W. REGEHR. 1994. Relationship between N_2-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. Can. J. Fish. Aquat. Sci. 51: 2254–2266.

FLETT, R.J., D.W. SCHINDLER, R.D. HAMILTON, AND N. CAMPBELL. 1980. Nitrogen fixation in Canadian Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 37: 494–505.

HAMILTON, A.L. 1991. The Stockholm Water Prize and the Experimental Lakes Area. Can. J. Fish. Aquat. Sci. 48: 1132–1135.

HANN, B.J., P.R. LEAVITT, AND P.S.S. CHANG. 1994. Cladocera community response to experimental eutrophication in Lake 227 as recorded in laminated sediments. Can. J. Fish. Aquat. Sci. 51: 2312–2321.

HENDZEL, L.L., R.E. HECKY, AND D.L. FINDLAY. 1994. Recent changes of N_2-fixation in Lake 227 in response to reduction of the N:P loading ratio. Can. J. Fish. Aquat. Sci. 51: 2247–2253.

JOHNSON, W.E., AND J.R. VALLENTYNE. 1971. Rationale, background, and development of experimental lake studies in northwestern Ontario. J. Fish. Res. Board Can. 28: 123–128.

KLING, H.J., D.J. FINDLAY, AND J. KOMÁREK. 1994. *Aphanizomenon schindleri* sp.nov.: a new nostocacean cyanoprokaryote from the Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 51: 2267–2273.

LEAVITT, P.R., AND D.L. FINDLAY. 1994. Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci. 51: 2286–2299.

LEAVITT, P.R., B.J. HANN, J.P. SMOL, B.A. ZEEB, C.E. CHRISTIE, B.WOLFE, AND H.J. KLING. 1994. Paleolimnological analysis of whole-lake experiments: an overview of results from Experimental Lakes Area Lake 227. Can. J. Fish. Aquat. Sci. 51: 2322–2332.

SCHINDLER, D.W. 1971. Carbon, nitrogen and phosphorus and the eutrophication of freshwater lakes. J. Phycol. 7: 321–329.

SCHINDLER, D.W., G.J. BRUNSKILL, S. EMERSON, W.J. BROECKER, AND T.-H. PENG. 1972. Atmospheric carbon dioxide: its role in maintaining phytoplankton standing crops. Science (Wash., D.C.) 177: 1192–1194.

SCHINDLER, D.W., R.H. HESSLEIN, AND M.A. TURNER. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish Aquat. Sci. 44 (Suppl. 1): 26–33.

WOLFE, B., H.J. KLING, G.J. BRUNSKILL, AND P. WILKINSON. 1994. Multiple dating of a freeze core from Lake 227, an experimentally fertilized lake with varved sediments. Can. J. Fish. Aquat. Sci. 51: 2274–2285.

ZEEB, B.A., C.E. CHRISTIE, J.P. SMOL, D.L. FINDLAY, H.J. KLING, AND H.J.B. BIRKS. 1994. Responses of diatom and chrysophyte assemblages in Lake 227 sediments to experimental eutrophication. Can. J. Fish. Aquat. Sci. 51: 2300–2311.

Recent Changes of N_2-Fixation in Lake 227 in Response to Reduction of the N:P Loading Ratio[1]

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Continuous artificial additions of nitrogen (N) and phosphorus (P) to Lake 227 at a loading ratio of 11:1 (atom:atom; 5:1 by mass) from 1975 to 1989, combined with natural inputs, resulted in variable mean annual total N and total P concentrations and changes in phytoplankton species composition. Summer phytoplankton occasionally shifted from N_2-fixing cyanobacteria to non-N_2-fixing forms. In 1990, additions of N fertilizer were stopped in order to test the hypothesis that low N:P loading rates cause selection for heterocystous cyanobacteria and stimulate N_2-fixation. Five years of phytoplankton nutrient-status measurements, together with estimates of N_2-fixation before and after lowering the N:P loading ratio, verified the importance of this ratio in producing blooms of N_2-fixing cyanobacteria. Nitrogen input via biological N_2 fixation increased, and summer dominance by heterocystous cyanobacteria returned as a consequence of lowering the N:P ratio of external inputs. Seasonally, N return from the epilimnetic sediments increased after a period of high planktonic N_2-fixation rates. This regenerated N reduced the abundance of heterocystous cyanobacteria and lowered late summer N_2-fixation rates. Over several years, preferential regeneration of N relative to P maintained the N:P ratio of the internally cycling nutrient pool, despite the reduction in the external N:P loading ratio.

Les apports artificiels et continus d'azote (N) et de phosphore (P) dans le lac 227 dans des proportions de 11:1 (en nombre d'atomes; 5:1 en masse) entre 1975 et 1989, combinés aux apports naturels, ont donné lieu à des concentrations annuelles moyennes en N total et en P total qui étaient variables ainsi qu'à des changements au niveau de la composition spécifique du phytoplankton. À l'occasion, il y avait un glissement dans les communautés phytoplanctoniques estivales qui passaient des cyanobactéries fixatrices de N_2 à des formes qui ne fixent pas le N_2. En 1990, on a mis un terme à l'ajout de N afin de vérifier l'hypothèse voulant qu'un faible rapport N:P de la charge favorise la sélection des cyanobactéries à hétérocystes et stimule la fixation du N_2. La mesure pendant 5 ans des éléments nutritifs utilisables par le phytoplankton, ainsi que des estimations de la fixation du N_2 avant et après l'abaissement du rapport N:P de la charge, ont permis de vérifier à quel point ce rapport est important pour la production des proliférations de cyanobactéries fixatrices de N_2. Les apports d'azote via la fixation biologique du N_2 se sont accrus et la dominance des cyanobactéries à hétérocystes durant l'été est réapparue par suite de l'abaissement du rapport N:P dans les apports externes. Suivant un cycle saisonnier, la remise en circulation du N provenant des sédiments épilimniques s'est accrue au terme d'une période durant laquelle les taux de fixation planctonique du N_2 ont été élevés. Le N_2 ainsi régénéré a conduit à une réduction de l'abondance des cyanobactéries à hétérocystes et à un abaissement des taux de fixation du N_2 vers la fin de l'été. Sur plusieurs années, la régénération préférentielle du N relativement au P a gardé à son niveau le rapport N:P de recirculation interne du pool des éléments nutritifs malgré une réduction du rapport N:P de la charge externe.

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Experimental eutrophication of Lake 227 is the longest-running experiment at the Experimental Lakes Area (ELA). Lake 227 was chosen in the late 1960s as the site to examine the role of carbon (C) in eutrophication (Schindler 1971). Beginning in 1969, the lake received nitrogen (N) and phosphorus (P) in amounts roughly equivalent to existing loadings to the Great Lakes; the N:P loading was 29:1 (atom:atom; 13:1 by mass) (Findlay et al. 1994). The fertilization scheme was maintained for 6 yr; during this period, the phytoplankton community was dominated by chlorophytes and other non-N_2-fixing species (Schindler 1974, 1975, 1977; Leavitt and Findlay 1994). When the experimental N:P loading ratio was decreased to 11:1 (atom: atom; 5:1 by mass) in 1975, while maintaining a constant experimental P loading rate, the heterocystous cyanobacterium *Aphanizomenon gracile* Lemmermann became the dominant species (Schindler 1977) and significant N_2 fixation was measured (Flett et al. 1980). The external loading ratio was maintained at 11:1 for the next 15 yr (1975–1989); during this period, the magnitude and duration of blooms of N_2-fixing cyanobacteria were variable, and there was a tendency for strong bloom years to be followed by weak bloom years (Leavitt and Findlay 1994). Such variability

[1]This paper is one of a series done in honor of the 25th anniversary of the Experimental Lakes Area.

is characteristic of cyanobacterial blooms in eutrophic lakes but it was particularly disconcerting in Lake 227 because external loading rates were relatively constant. It was hypothesized that many years of N addition and N_2-fixation had saturated the sediments sufficiently that sediment return enhanced available N and reduced the selective advantage of N_2-fixing cyanobactaria (Schindler et al. 1987; Levine and Schindler 1992). However, other interpretations were also possible. For example, iron, molybdenum, and other trace elements essential for cyanobacterial growth may have become limiting (Horne and Commins 1987; Rueter and Petersen 1987; Wurtsbaugh 1988; Howarth and Marino 1990), or excess P may have been unavailable to trigger cyanobacterial blooms (Schindler 1977; Smith 1990). These possibilities were examined in mesocosm experiments in Lake 227 (R.E. Hecky and L.L. Hendzel, unpublished data), which led to the hypothesis that preferential N regeneration from epilimnetic sediments could produce an actual loading ratio to the epilimnion higher than expected from the external loading ratio. This higher-than-anticipated ratio could vary from year to year, depending on actual internal regeneration, and could cause the observed variability in cyanobacterial blooms.

This hypothesis was tested by ceasing the experimental loading of N in 1990 while maintaining P additions at the previous levels. It was expected that this would strongly favor N_2-fixing cyanobacteria and lead to predictable blooms of heterocystous cyanobacteria. The results of that experiment are the subject of this paper.

Methods

Site Description

Lake 227 is a small (5.0 ha) lake situated on the Canadian Shield of northwestern Ontario (Brunskill and Schindler 1971). The lake has a mean depth of 4.4 m and a maximum depth of 10.0 m. Vernal and autumnal mixing are usually incomplete and variable; stratification during the summer is well defined at 2–4 m, and the water below the thermocline is anoxic. The lake was fertilized with sodium nitrate ($NaNO_3$) and sodium hypophosphate (Na_2HPO_4) in 1969, and $NaNO_3$ and phosphoric acid (H_3PO_4) from May to October, 1970–1989 (Cruikshank 1991). The atomic and mass ratios of N:P in artificial loadings from 1969–1974 were 29:1 and 13:1, respectively; from 1975–1989 they were 11:1 and 5:1, respectively. Nitrogen additions (0.236 $mol \cdot m^{-2} \cdot y^{-1}$) were terminated beginning in 1990, and only H_3PO_4 was added in the same amount as in previous years (0.018 $mol \cdot m^{-2} \cdot yr^{-1}$).

Sampling Routine and Procedures

Lake 227 was sampled at least weekly for chlorophyll, particulate C (PC), particulate N (PN), particulate P (PP), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total nitrogen (TN = TDN + PN), and total P (TP = TDP + PP) between 1988 and 1991 and every 2 wk in 1992. In addition, phytoplankton nutrient deficiency indicators were measured over the ice-free period four times in 1988, five times in 1989, eight times in 1990 and 1991, and 12 times in 1992.

Particulate C, PN, and PP samples were filtered onto Whatman GF/C filters preignited at 500°C, and were stored frozen for later analysis. Chlorophyll *a* samples were collected on GF/C filters, stored frozen, extracted with methanol, and determined fluorometrically. Filtrate was analyzed the same day for TDN and TDP. All chemical analyses followed the methods of Stainton et al. (1977).

Particulate alkaline phosphatase activity and N-debt measurements followed the method of Healey and Hendzel (1980). N_2 fixation estimated from nitrogenase activity was measured using modifications of the acetylene reduction method described by Flett et al. (1976). All laboratory incubations were done in 50-cm^3 glass syringes fitted with three-way polycarbonate valves, at a light intensity of 80–100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (E = Einsteins) and a temperature of 20°C for 4 h. Following incubation and gas stripping, the syringes were placed upright on their plunger ends and allowed to sit for 1–2 min to settle all gas bubbles. The volume of the gas phase of each syringe was estimated; the syringe was then carefully inverted (valve pointing down), the valve was opened and the liquid phase was displaced by gently pushing downward on the plunger. The valve was closed when all liquid was expelled. To terminate any biological activity in the small amount of water remaining in each syringe, 1 mL of 1% hydrochloric acid was injected into each syringe. The valve was closed and the syringe was stored until gas analysis by flame ionization detection gas chromatography was performed (Hewlett-Packard Model 5750). During 1992, N_2 fixation incubations were done in a light gradient at in situ temperatures. All fixation determinations were performed in triplicate, and included a measurement of fixation in the dark along with a blank. All laboratory light measurements were made with a Biospherical Instruments QSL-100 light meter.

In 1992, estimates of nutrient regeneration from epilimnetic sediments were measured on eight occasions using 25-L transparent (light) and opaque (dark) plexiglass boxes (Ramlal et al. 1994). The boxes were gently set into the epilimnetic sediments of Lake 227 (~1 m depth) by skin diving and left in place from 24–48 h. Water within the boxes was gently stirred by a battery powered stirring bar (1 rpm). The boxes were sampled at various time intervals by drawing water into 50-cm^3 syringes through a connecting length of Tygon tubing, the upper end of which was floating at the water surface. Two 100-mL volumes of water were discarded before one glass syringe was filled from each box and fixed for dissolved oxygen determinations. Three additional water samples were taken from each box for TDP, nitrate, nitrite, ammonium, pH, and dissolved inorganic C determinations.

Results and Discussion

Lake Nutrient Status

Five years of seston composition ratios for Lake 227 indicated that the phytoplankton were P deficient in all years (Fig. 1). However, 1990, the first year of reduced N loading, was a transitional year characterized by lower P deficiency than in the other years. Ratios of C:P and N:P (Figs. 1a and 1b) were generally severely P deficient, whereas C:N (Fig. 1c) was only moderately N deficient for all years except 1990 when C:N was at or near severely N deficient on several sampling dates.

Metabolic indicators also showed that Lake 227 was usually P deficient (Fig. 2). Alkaline phosphatase activity (Fig. 2a) was lower in 1988, 1989, and 1991 than in 1990 and 1992 but still indicated severe P deficiency for all years. Nitrogen debt (Fig. 2b) and N_2-fixation (Fig. 2c) rates were

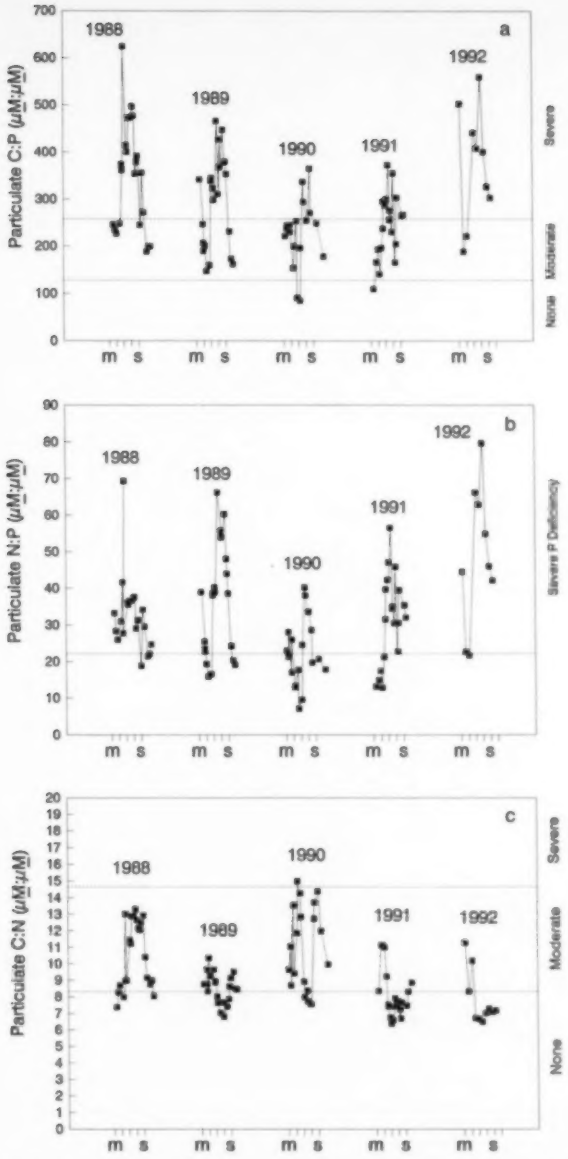

FIG. 1. Seasonal particulate compositional ratios (atom:atom) for Lake 227 from May (m) through September (s) from 1988–92 for: (a) carbon (C) : phosphorus (P); (b) nitrogen (N) : P; and (c) C:N. Levels of nutrient deficiency are identified.

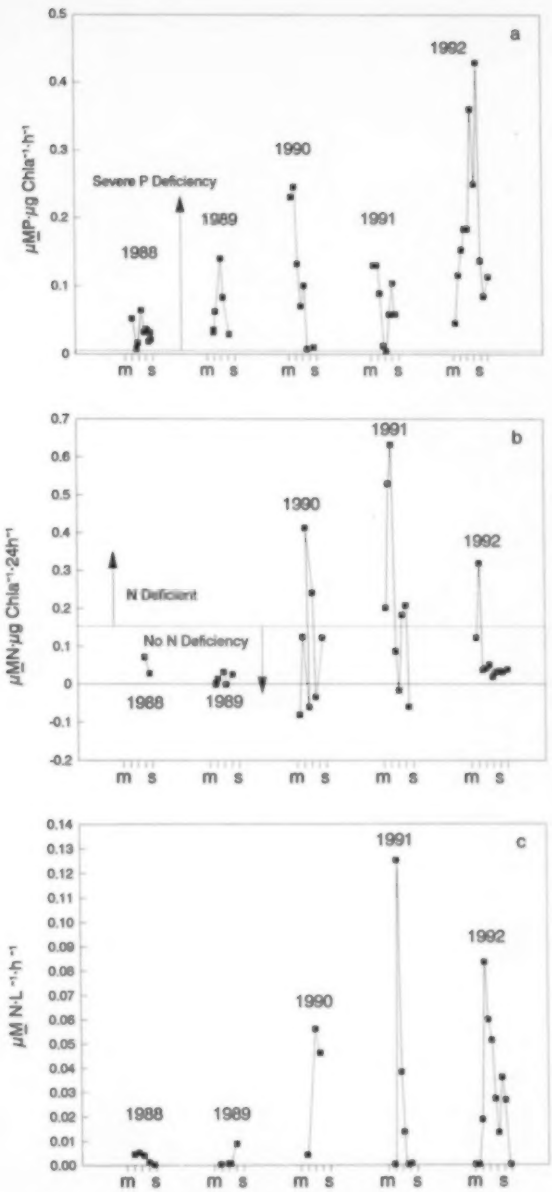

FIG. 2. Seasonal molar rates of: (a) particulate alkaline phosphatase activity; (b) nitrogen (N) debt; and (c) N_2-fixation for Lake 227 from May (m) through September (s) from 1988–92. Chl *a* = chlorophyll *a*.

low in 1988 and 1989, and there was evidence of greater deficiency in 1991 and 1992 than indicated in Fig. 1c. Beginning in 1990, when additions of N fertilizer were stopped, alkaline phosphatase activity, N debt, and N_2-fixation increased. Both particulate alkaline phosphatase activity and N debt achieved values indicative of severe P and N deficiency, respectively. Significant N debt occurred at least once in each of the last 3 yr, but alkaline phosphatase rates indicated severe P deficiency throughout each year. High rates of N_2 fixation were measured following periods of N debt from 1990–1992.

Sediment Regeneration of N

Gross and net epilimnetic sediment flux rates of N and P followed a seasonal pattern, but the N and P fluxes were not synchronous with each other (Fig. 3). The gross return of TDP (i.e., dark box, without the demands of photosynthesis; Fig. 3a) declined to zero by mid-June, remained unchanged to late July, and sharply increased in August. Gross return of dissolved inorganic nitrogen (DIN) (Fig. 3b) was low during early June, increased to a maximum by the

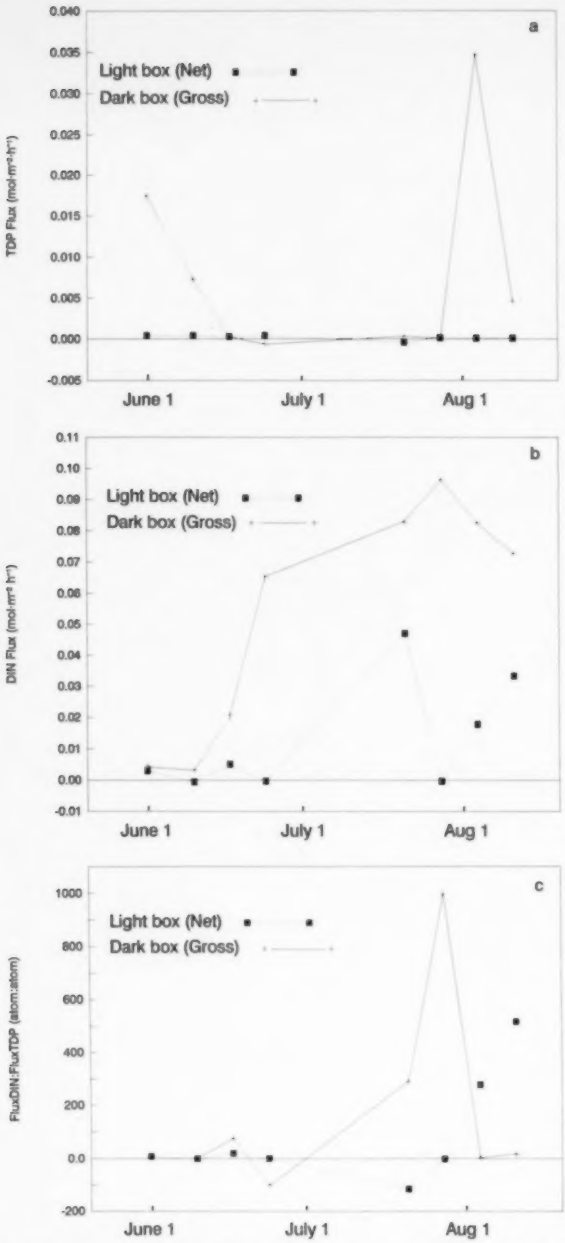

FIG. 3. Fluxes of: (a) total dissolved phosphorus (TDP); (b) dissolved inorganic nitrogen (DIN = NO_2 + NO_3 + NH_4); and (c) DIN:TDP from the epilimnetic sediments of Lake 227 for June–August 1992. Results are expressed as hourly rates from measurements over 24 h (light boxes) and ~13.5 h (dark boxes).

end of July, and then decreased slightly into August. A low (<22:1) gross DIN:TDP flux ratio (Fig. 3c) in early June and early August bracketed a higher ratio (>22:1) from the beginning of July to the beginning of August.

The daily net return of TDP at 1 m depth (i.e., light box, incorporating photosynthetic influences; Fig. 3a) remained constantly low through the summer. This trend was interrupted by a negative value (net daily uptake) on July 21. In contrast to the flux of P, the net flux of DIN from the sediments (Fig. 3b) was low until the end of June, and then increased toward the end of July and the beginning of August, but was interrupted by a zero value on July 28. The ratio of the net fluxes of DIN:TDP (Fig. 3c) increased sharply to >500:1 after July 28. This increase followed the seasonal peak in N_2-fixation.

Seasonal Dynamics

The nutrient status of the lake phytoplankton from 1988–1989, as illustrated by compositional ratios (Fig. 1) and metabolic indicators (Fig. 2), suggested severe P deficiency. The community at that time was a mixture of N_2-fixing and non-N_2-fixing algae (Findlay et al. 1994); N_2 fixation was low. Following the elimination of experimental N loading and the resultant lowering of the experimental N:P ratio in 1990, N_2-fixing cyanobacteria consistently dominated in Lake 227. This community was P deficient as a consequence of increased N_2-fixation and efficient N recycling. Cyanobacterial dominance and periods of N_2-fixation (Fig. 4) were preceded by pulses of N deficiency. The seasonal cycle for the years following termination of N fertilization (1990–1992) began with a non-N_2-fixing, P-limited spring population. Phosphorus limitation was relieved by the experimental addition of P, which induced a brief period of N deficiency. This was followed by the onset of a bloom of heterocystous N_2-fixing cyanobacteria and a resultant increase in N_2-fixation, total biomass, and cellular N:P ratios (Figs. 4a–4c). The increase in the compositional N:P ratio, after the peak in N_2-fixation, was especially apparent in 1991 and 1992. Nitrogen biologically fixed by heterocystous cyanobacteria was deposited on epilimnetic sediments, where N was selectively regenerated (relative to P). This regenerated N was available to all algal groups, and the heterocystous bloom was succeeded by other algal groups.

Input to Lake 227 of fixed elemental N, based on seasonal open-water, time-weighted N_2-fixation data amounted to ~500 mol N in 1988 and 1989, whereas ~7–10 times more was added in 1990, 1991, and 1992 (Table 1). In addition, time-weighted estimates of gross N and P released from the epilimnetic sediments for the 3-mo period of maximum productivity (June–August) in 1992 amounted to ~2600 mol N and ~140 mol P (Table 2). The N:P ratio of this gross return would be sufficient to sustain high biomasses. Similar estimates of net N and P release indicated that ~60% of the N and 97% of the P was effectively retained in the epilimnetic sediments. The relatively high P retention by the benthic microbial community implies P limitation of bacterial growth as well as algal growth. It also imposes a very high N:P ratio on the regenerated nutrients returned to phytoplankton in the mixed layer. This high N:P ratio would not be selectively advantageous for heterocystous cyanobacteria because species that use P efficiently would be favored.

The maximum seasonal peak DIN:TDP ratio in 1992, calculated on the gross fluxes of N and P (Fig. 3c), was preceded by the cyanobacterial bloom (Fig. 4c). Initial fluxes (beginning of June) preceded the period of maximum N_2-fixation and returned less N relative to P (low DIN:TDP), which reflected the experimental design (P only addition) and favored the initiation of the N_2-fixer bloom. Once the

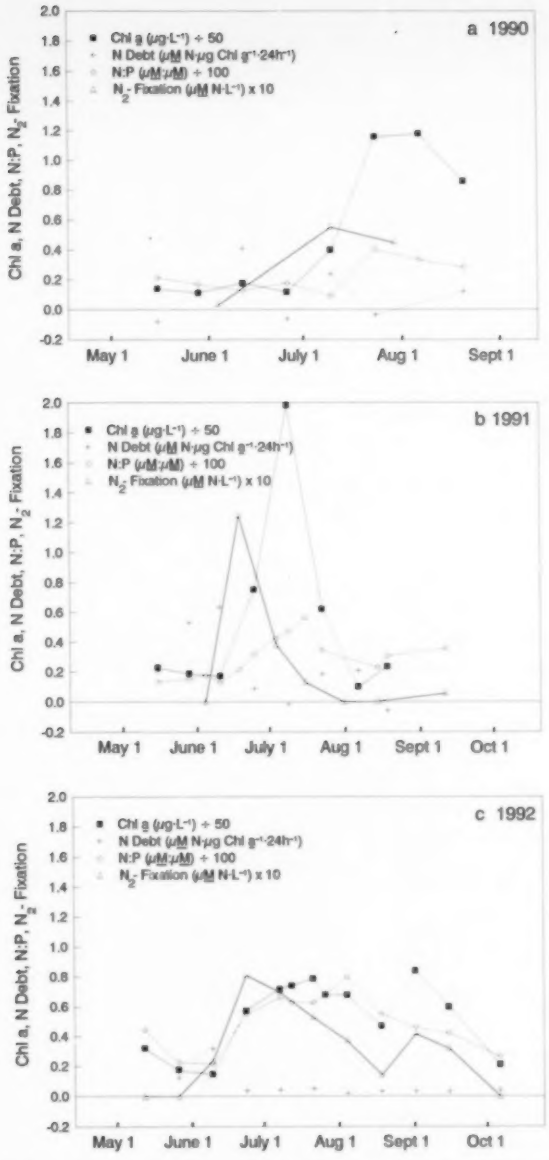

FIG. 4. Seasonal changes in chlorophyll *a* (Chl *a*; $\mu g \cdot L^{-1}$), nitrogen (N) debt ($\mu M\ N \cdot \mu g\ Chl\ a^{-1} \cdot 24\ h^{-1}$, N:P ($\mu M:\mu M$), and N_2-fixation ($\mu M\ N \cdot L^{-1}$) for Lake 227 for (a) 1990; (b) 1991; and (c) 1992.

bloom became established (high N_2-fixation rates), a period followed when the N:P ratio of regenerated nutrients increased rapidly, reaching a maximum on July 21. This coincided with the period of maximum P deficiency (i.e., high N:P season ratios, Fig. 3c). A reduction in the sedimentary DIN:TDP flux ratio in August (Fig. 3c) seemed to be associated with a smaller, secondary peak of N_2-fixation in September (Fig. 4c).

The actual sedimentation of N and P mirrored the cyanophyte bloom and the observed return of N and P from the epilimnetic sediments. The monthly average N:P ratios

TABLE 1. Estimated open-water, time-weighted nitrogen (N) input to Lake 227 by N_2-fixation for 1988–1992.

Year	Interval	mol N · yr^{-1}
1988	May 01 – Sept. 01	449.8
1989	May 01 – Sept. 01	576.1
1990	May 01 – Sept. 01	4764.8
1991	May 01 – Sept. 12	3567.5
1992	May 01 – Oct. 07	5354.5

of sedimenting seston for June, July, and August were 20.32, 26.11, and 23.51 (atom:atom), respectively. The low N:P ratio of the sedimenting seston was also indicative of the selective regeneration of N that was characteristic of the epilimnetic sediments. Season N:P ratios (mol:mol) in the mixed layer averaged >20 and exceeded 50 during blooms; for each mole of P sedimented, a minimum of 10 moles were regenerated. It is also clear that the natural flux of N exceeded the experimental loadings of N in all years. These large and variable internal and external fluxes of N, compared with the experimental flux, accounted for the variability in the strength and duration of the heterocystous cyanobacteria blooms from year to year (Findlay et al. 1994). Calculated N and P gross returns to the surficial waters from epilimnetic sediments for June, July, and August 1992 were three times greater than experimental additions of N in 1988 and 1989. Clearly, the internal supply of N to the epilimnion was a dominant flux even as N was being added.

Benthic retention of P occurred in the light boxes, indicating a photosynthetic demand that competed with the pelagic phytoplankton for P. In contrast, there was excess N relative to benthic photosynthesis, resulting in return of N to the mixed layer. This return eventually allowed other non-N_2-fixing algal groups to successfully compete for P on the seasonal time scale. The greater net release of N than P over the season had more of an influence on the physiological indicators of phytoplankton N deficiency (Fig. 4c), than on P deficiency (Figs. 1a and 2a), which was always severe in Lake 227. When N deficiency did occur in the late spring, the phytoplankton responded by increasing N_2-fixation, which eliminated the deficiency. In previous years, when the external loading ratio was 11:1 (atom:atom), N fixed biologically during strong bloom years may have affected subsequent years through the internal regeneration pathway, producing the observed annual high variance in bloom formation (Findlay et al. 1994). When experimental loading of N ceased (1990), annual external loading of P overwhelmed the supply of N available from sediment regeneration. Consequently, heterocystous cyanobacteria became highly favored and they should regularly dominate during the summer in Lake 227.

Interannual Dynamics

Mean annual, open-water, epilimnetic TN:TP ratios from 1975–1992 indicated that Lake 227 had fixed and retained varying amounts of N relative to added P (Findlay et al. 1994). For example, estimates of N_2-fixation based on heterocyst numbers ranged from 500 $mol \cdot m^{-2} \cdot yr^{-1}$ in 1978 to 5000 $mol \cdot m^{-2} \cdot yr^{-1}$ in 1992. Through the mid-1980s, changes in the biomass and intensity of the N_2-fixing cyanophyte blooms were reflected by changes in the TN:TP ratios.

TABLE 2. Seasonal inputs and losses of nitrogen (N) and phosphorus (P) to the Lake 227 epilimnion (epi.) during May, June, July, and August, 1988–1992. Estimates are corrected for morphology (0–2.5 m). nd = Not done.

	Inputs (mol)								Losses (mol)	
Year	Precip N	Precip P	Runoff N	Runoff P	Fertilizer N	Fertilizer P	Epi. sediment N flux (gross)	Epi. sediment P flux (gross)	N_2-fixation	Sedimentation losses of N	Sedimentation losses of P
1988	2333.12	15.26	1098.74	7.58	874.71	734.87	nd	nd	449.78	nd	nd
1989	2021.13	15.48	1758.41	14.29	874.71	734.87	nd	nd	576.14	nd	nd
1990	968.09	11.71	1359.32	10	0	734.87	nd	nd	4764.76	nd	nd
1991	814.59	12	837.44	7.61	0	734.87	nd	nd	3567.5	nd	nd
1992	1792.68	30.52	1659.88	12.77	0	734.87	2606.3	139.85	5354.47	11 396.38	489.34

Relative abundance of the N_2-fixers (Findlay et al. 1994) increased significantly after 1988, a year when the lake produced a N_2-fixer bloom of short duration and was dominated by chlorophytes instead. The biomass of the N_2-fixers, expressed as a percentage of the total biomass (Findlay et al. 1994), reflected a similar increase through time; at times over the last two years, it comprised >90% of the total biomass. Variable return of N from the sediments (Schindler et al. 1987; Levine and Schindler 1992), together with equally variable additions from surface runoff, precipitation, and N_2-fixation (Table 2) probably all contributed to this non-steady-state condition. The significant bloom of N_2-fixers in 1989 (Findlay et al. 1994), the year prior to total elimination of N fertilization, was consistent with this non-steady state. This bloom occurred after a period of decline of the TN:TP (Findlay et al. 1994). However, the relative abundance of N_2-fixers, as a percentage of total biomass, increased each year following the change in loading. In contrast, by the early 1980s sodium, and to some degree P, had reached a steady state relative to the experimental loadings (Schindler et al. 1987).

N_2 fixation and the N flux from the epilimnetic sediments add significant amounts of N into the epilimnion of the lake; therefore, it is not surprising that it is difficult to predict the abundance of N_2-fixing cyanobacteria from external loading rates alone. In the long term, the complexities of the N cycle, which produces and consumes gaseous forms of N in exchange with the atmosphere, will generate a variable steady state that depends on the comparative strength of the internal cycle, the N:P ratio of the cycle, and the N:P ratio of external sources.

Experimental eutrophication of Lake 227 has involved three different N:P ratios, but P loading has been kept constant. Each reduction in the ratio caused blooms of heterocystous cyanobacteria. The original reduction from 29:1 to 11:1 created a combination of internal and external loading that required variable amounts of fixed N to meet the N deficit created by the change in external loading. The internal ratio remained high, and the internal flux was variable but significant relative to the external load. The result was high interannual variability of the extent of heterocystous cyanobacterial blooms and biological N_2-fixation. Heterocystous cyanobacterial blooms became necessary to meet the N deficit in Lake 227 and thus were less variable. The predictability of heterocystous cyanobacteria blooms in other lakes will likewise depend on the relative magnitudes of the internal and external fluxes and their N:P ratios.

Over the past 22 yr, the lake used N_2-fixation as one pathway to meet the N requirements of phytoplankton (Findlay et al. 1994). Reducing experimental N loading to zero substantially increased the demand for fixed N and the actual amounts of N fixed (Table 2). Previous enclosure experiments (Levine and Schindler 1992; Levine 1983) also showed that N_2-fixation was stimulated by low N:P external loading ratios. Both of these results confirm earlier conclusions about how ELA lakes compensated for N shortages at low N:P supply ratios (Schindler 1977; Flett et al. 1980; Howarth et al. 1988). Furthermore, littoral mesocosm experiments (Levine and Schindler 1992) showed that sediments of artificially fertilized Lake 303 returned considerable quantities of NH_4 but essentially no P to the water column. In Lake 227, measured sediment fluxes (Table 2; Figs. 3a and 3b) demonstrated that a portion of this fixed N enters back

into the internal-cycling N pool. The phytoplankton of the mixed layer have to integrate external and internal nutrient sources of variable stoichiometry and create cells of relatively constrained stoichiometry (Hecky and Kilham 1988). Nutrient return across the thermocline during summer is negligible (Quay et al. 1980), and N_2-fixation is favored if external N loading is inadequate, which keeps the lake in a P-limited state and supports high annual biomasses proportional to inputs (Schindler 1977). At high N:P loading ratios, heterocystous cyanobacteria get no selective advantage from their N_2-fixing capability, whereas at quite low ratios they will be favored. Blooms will be least predictable at intermediate ratios.

Acknowledgments

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References

Brunskill, G.J., and D.W. Schindler. 1971. Geography and bathymetry of selected lake basins. Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139–155.

Cruikshank, D.R. 1991. Whole lake additions in the Experimental Lakes Area, 1986–1989. Can. Data Rep. Fish. Aquat. Sci. 816: 18 p.

Findlay, D.L., R.E. Hecky, L.L. Hendzel, M.P. Stainton, and G. Regehr. 1994. Relationships between N_2-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. Can. J. Fish. Aquat. Sci. 51: 2254–2266.

Flett, R.J., R.D. Hamilton, and N.E.R. Campbell. 1976. Aquatic acetylene-reduction techniques: solutions to several problems. Can. J. Microbiol. 22: 43–51.

Flett, R.J., D.W. Schindler, R.D. Hamilton, and N.E.R. Campbell. 1980. Nitrogen fixation in Canadian Precambrian Schield lakes. Can. J. Fish. Aquat. Sci. 37: 494–505.

Healey, F.P., and L.L. Hendzel. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Can. J. Fish. Aquat. Sci. 37: 442–453.

Hecky, R.E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. Limnol. Oceanogr. 33: 796–822.

Horne, A.J., and M.L. Commins. 1987. Macronutrient controls on nitrogen fixation in planktonic cyanobacterial populations. N.Z. J. Mar. Freshwater Res. 21: 413–423.

Howarth, R.W., and R. Marino. 1990. Nitrogen-fixing cyanobacteria in the plankton of lakes and estuaries: a reply to the comment by Smith. Limnol. Oceanogr. 35: 1859–1863.

Howarth, R.W., R. Marino, and J.J. Cole. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. Limnol. Oceanogr. 33: 688–701.

Leavitt, P.R., and D.L. Findlay. 1994. Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lake Area, Ontario. Can. J. Fish. Sci. 51: 2286–2299.

Levine, S.N. 1983. Natural mechanisms that ameliorate nitrogen shortages in lakes. Ph.D. thesis, University of Manitoba, Winnipeg.

Levine, S.N., and D.W. Schindler. 1992. Modification of the N:P ratio in lakes by in situ processes. Limnol. Oceanogr. 37: 917–935.

Quay, P.D., W.S. Broecker, R.H. Hesslein, and D.W. Schindler. 1980. Vertical diffusion rates determined by tritium tracer experiments in the thermocline and hypolimnion of two lakes. Limnol. Oceanogr. 25: 201–218.

Ramlal, P.S., R.H. Hesslein, R.E. Hecky, E.J. Fee, J.W.M. Rudd, and S.J. Guildford. 1994. The organic carbon budget of a shallow Arctic tundra lake on the Tuktoyaktuk Peninsula, N.W.T., Canada. Biochemistry 24: 145–172.

Rueter, J.G., and R.R. Petersen. 1987. Micronutrient effects on cyanobacterial growth and physiology. N.Z. J. Mar. Freshwater Res. 21: 435–445.

Schindler, D.W. 1971. Carbon, nitrogen and phosphorus and the eutrophication of freshwater lakes. J. Phycol. 7: 321–329.

Schindler, D.W. 1974. Experimental studies of eutrophication and lake recovery: some implications for lake management. Science (Wash., D.C.) 184: 897–899.

Schindler, D.W. 1975. Whole lake eutrophication experiments with phosphorus, nitrogen and carbon. Verh. Int. Ver. Theor. Angew. Limnol. 19: 3221–3231.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. Science (Wash., D.C.) 195: 260–262.

Schindler, D.W., R.H. Hesslein, and M.A. Turner. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish. Aquat. Sci. 44(Suppl 1): 26–33.

Smith, V.H. 1990. Nitrogen, phosphorus, and nitrogen fixation in lacustrine and estuarine ecosystems. Limnol. Oceanogr. 35: 1852–1859.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. The chemical analysis of fresh water. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

Wurtsbaugh, W.A. 1988. Iron, molybdenum and phosphorus limitation of N_2 fixation maintains nitrogen deficiency of plankton in the Great Salt Lake drainage (Utah, USA). Verh. Int. Ver. Theor. Angew. Limnol. 23: 121–130.

Relationship Between N_2-Fixation and Heterocyst Abundance and its Relevance to the Nitrogen Budget of Lake 227[1]

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A significant relationship between seasonal N_2-fixation rate and heterocyst abundance has been found in Lake 227 and can be modelled to estimate areal rates of N_2-fixation based on the known historical phytoplankton records. Experimental imposition of low nitrogen (N): phosphorus (P) loading ratios stimulated N_2-fixation. The N:P molar ratio of the internal pool of nutrients oscillates around a mean of 30:1, despite experimental loading ratios that vary from 13:1 to 0. Maintenance of this internal nutrient mass ratio requires preferential regeneration of N through time at all loading rates. Heterocystous, cyanobacteria blooms are most predictable during imposition of a very low external loading N:P ratio and high P loading relative to natural sources. Knowledge of the internal loading N:P ratio and relative contribution of internal and external loadings are required for predicting blooms at intermediate external N:P loading rates. The internal N:P ratio has fluctuated within narrow limits over 25 yr of nearly constant P loading because of adjustments in N_2-fixation, N sedimentation, and denitrification, which have counterbalanced changing experimental N loading regimes.

Il existe un rapport significatif entre le taux saisonnier de fixation de l'azote et l'abondance des hétérocystes dans le lac 227. Ce rapport peut être modélisé pour fournir une estimation des taux par unité de surface de la fixation de l'azote gazeux, à partir des relevés historiques sur le phytoplancton. La fertilisation expérimentale du lac à un faible rapport N:P a stimulé la fixation du N_2. Dans le pool interne des éléments nutritifs, le rapport molaire N:P demeure aux environs de 30:1 malgré la fertilisation expérimentale à des facteurs N:P variant entre 13:1 et 0. Pour que soit maintenu ce rapport massique interne des éléments nutritifs, il faut une régénération préférentielle de l'azote au fil du temps, peu importe les facteurs de charge. Les proliférations de cyanobactéries à hétérocystes sont le plus prévisibles lorsqu'on procède à une fertilisation à très faible rapport N:P et que la charge en P est élevée par rapport aux sources naturelles. Il faut connaître le rapport N:P des apports internes ainsi que la contribution relative des apports internes comme des apports externes pour prévoir les proliférations algaires lorsque les apports externes se font à des taux N:P intermédiaires. Pendant les 25 ans que l'apport en P a été presque constant, le taux N:P de l'apport interne a fluctué à l'intérieur d'étroites limites sous le jeu de la fixation du N_2, de la sédimentation du N et de la dénitrification, qui ont fait contrepoids aux régimes expérimentaux et variables d'apport de N.

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Filamentous cyanobacterial blooms in fresh water can cause unwanted water quality effects such as fish kills, beach closures, and increased costs of drinking water treatment. Many hypotheses have been presented to explain these blooms: elevated water temperature (Robarts and Zohary 1987; McQueen and Lean 1987; Tilman et al. 1986), low light levels (Zevenboom and Mur 1980), increased zooplankton grazing (Haney 1987), buoyancy of filamentous cyanobacteria (Klemer et al. 1982; Paerl and Ustach 1982), low carbon dioxide (CO_2) levels and high pH values (King 1970; Shapiro 1990), micronutrients (Reuter and Petersen 1987), and low nitrogen (N): phosphorus (P) ratios (Flett et al. 1980; Howarth et al. 1988a, 1988b; Levine and Schindler 1992; Smith 1983, 1990).

Loading rates and ratios of nutrient inputs are major factors inducing cyanobacterial blooms (Schindler 1974, 1977; Holmgren 1983; Levine and Schindler 1992). Schindler (1974, 1977) demonstrated, through artificial eutrophication of the north basin of Experimental Lakes Area (ELA) Lake 226, that P is the essential nutrient limiting algal biomass. The lake was separated into two basins with a vinyl-reinforced curtain, both basins received carbon (C) and N, but the north basin also received P at a N:P ratio of 5:1 (by weight). A cyanobacterial bloom immediately formed in the north basin and was dominated by heterocystous *Anabaena spiroides* Klebs. It was hypothesised that increased P loading, at a low N:P ratio, caused the phytoplankton to become N deficient, thus giving heterocystous cyanobacteria a competitive advantage over other phytolankton because of their ability to fix atmospheric N. Because the cyanobacteria met the N requirement of the phytoplankton community by fixation, algal biomass remained P-limited. To test this hypothesis, the loading rate of N:P in Lake 227 was reduced from 13:1 (1969–1974) to 5:1 in 1975 (Table 1). *Aphanizomenon gracile* Lemmermann, a heterocystous cyanobacteria, responded

[1]This paper is one of a series done in honor of the 25th anniversary of the Experimental Lakes Area.

TABLE 1. Annual source inputs and ratios of nitrogen (N) and phosphorus (P) to Lake 227 from 1970 to 1992. Fertilizer and precipitation inputs are direct measurements. Inputs from runoff are estimates based on the Lake 239 northwest inflow. N_2-fixation estimates are from the model referred to in the text. Where available, actual N_2-fixation measurements are in parentheses. The total N (TN):total P (TP) loading ratios are calculated from TN inputs minus N_2-fixation inputs/TP inputs.

Year	N Fertilizer (kg) A	N Precip. (kg) B	N Runoff (kg) C	N_2-Fixation (kg) D	% contributed by N_2-fixation D/(A+B+C+D)	P Fertilizer (kg)	P Precip. (kg)	P Runoff (kg)	Fertilizer N:P ratio (kg/kg)	Precip. + Runoff N:P ratio (kg/kg)	TN:TP Loading ratio (kg/kg)
1970	315	26	10	0	0	24	1.32	0.96	13	15	13.4
1971	315	25	34	0	0	24	2.04	2.29	13	14	13.2
1972	315	31	25	0	0	24	1.02	0.74	13	31	14.4
1973	315	21	19	0	0	24	1.24	0.56	13	22	13.8
1974	315	24	23	0	0	24	1.12	0.77	13	25	13.6
1975	112	23	19	12	4	23	0.81	0.47	5	33	6.3
1976	112	24	15	10	6	23	0.65	0.37	5	37	6.3
1977	112	25	25	20	11	23	1.02	0.59	5	32	6.6
1978	112	23	26	7	4	23	0.77	0.59	5	36	7.0
1979	112	22	17	49	24	23	0.96	0.34	5	30	6.2
1980	112	23	26	30	16	23	0.71	0.40	5	44	6.3
1981	112	22	34	17	9	23	0.77	0.50	5	45	6.5
1982	112	33	30	77	31	23	0.84	0.87	5	37	7.1
1983	112	34	16	20	11	23	0.68	0.53	5	41	6.7
1984	112	41	22	26	13	23	0.74	0.47	5	52	7.2
1985	112	49	56	16	7	23	1.12	1.80	5	36	8.4
1986	112	38	22	24	13	23	0.68	0.87	5	39	7.0
1987	112	34	18	21	11	23	0.40	0.34	5	70	6.9
1988	112	48	33	15 (6.30)	7	23	0.68	0.84	5	53	7.9
1989	112	45	38	29	10	23	0.84	0.99	5	46	7.9
1990	0	21	20	46 (66.74)	52	23	0.53	0.37	0	46	1.7
1991	0	31	34	39 (49.97)	37	23	0.74	0.68	0	45	2.7
1992	0	40	54	68 (75.00)	42	23	1.39	1.15	0	37	3.7

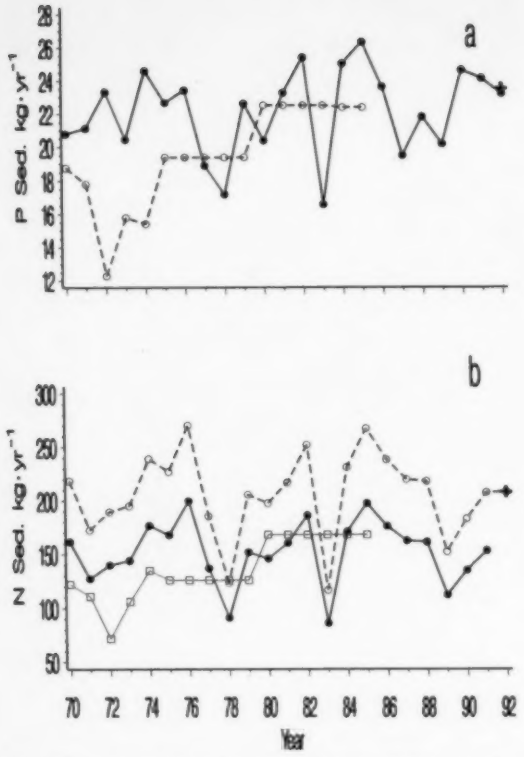

FIG. 1. Comparison of sedimentation losses of (a) phosphorus (P) and (b) nitrogen (N) to Lake 227 from 1970 to 1992 calculated from different data sources. (a) The solid circles are calculated from the P budget; the open circles are calculated from sediment core data (Wolfe et al. 1994); (b) The solid circles are calculated from the P budget and sediment N:P ratio; the open circles are calculated from the P budget and seston N:P ratio; and the open squares are calculated from sediment core data (Wolfe et al. 1994). The plus sign in (a) and (b) is an estimate based on sediment traps at 6 m depth (J. Elser, Arizona State University, Tempe, Ariz., unpublished data).

immediately with increased biomass. Flett et al. (1980) observed no N_2-fixation in Lake 227 from 1972 to 1974, but concluded that N_2-fixation in Lake 227 accounted for as much as 19% of the annual N inputs in 1975 after the N:P loading ratio was lowered to 5:1.

N_2-fixation occurs within the heterocysts of nostocaceaen cyanobacteria (Stewart et al. 1967, 1968). The existence of a relationship between N_2-fixation and heterocyst abundance has been documented (Stewart et al. 1967, 1968; Rönicke 1986; Levine and Lewis 1987). We present the relationship of N_2-fixation to heterocyst abundance for Lake 227, and we use historic heterocyst abundances to infer the annual contribution of cyanobacterial N_2-fixation to the total N budget of this lake, which has been artificially eutrophied for the past 23 yr. The estimation of N_2-fixation over many years allows the internal dynamics of N and P cycling to be followed, given the experimental design used and the approaches taken. Cessation of experimental N loading in 1990 also allowed a further test of the hypothesis that N:P loading ratios control the intensity and duration of N_2-fixing filamentous cyanobacteria. Hendzel et al. (1994) address the seasonal dynamics of heterocystous cyanobacteria blooms in Lake 227, and the response of these cyanobacteria to the elimination of experimental N loading.

Study Site

The ELA is located in northwestern Ontario (93°41′W 49°41′N) on the southwestern edge of the Canadian Shield. The watershed surrounding Lake 227 has a typical mature boreal forest growth. The lake has a surface area of 5 ha, a maximum depth of 10 m, and a mean depth of 4.4 m. There is no discrete inflowing stream and the single outflowing stream is the only water loss from the basin other than evaporation. Lake 227 in its natural state was chemically typical of Canadian Shield lakes in the area, but had exceptionally low concentrations of dissolved inorganic C (DIC) (Schindler et al. 1973). Thermal stratification occurs in early May and lasts until late September turnover, but complete water column mixing in spring and fall is rare.

In 1969, Lake 227 was selected as the site of an experiment to test the hypothesis that inorganic C availability could limit phytoplankton growth (Schindler 1974, 1975). Phosphorus was added to the epilimnion as phosphoric acid at the rate of 0.48 $g \cdot m^{-2} \cdot yr^{-1}$; N was simultaneously added as sodium nitrate ($NaNO_3$) at an N:P ratio of 13:1 (by weight). In 1975, the loading ratio to Lake 227 was decreased to 5:1 to test the hypothesis that N_2-fixing species would be competitively favoured and would meet the N deficit to maintain P limitation of algal biomass (Schindler 1977). The loading regime was maintained at 5:1 until 1990, when the addition of N ceased (Table 1); however, P additions were continued at previous levels (Hendzel et al. 1994).

Methods

Phytoplankton and Heterocyst Enumeration

The phytoplankton in Lake 227 were sampled every second week from ice out until after thermal destratification from 1969–1992. Integrated samples for both phytoplankton and water chemistry were obtained from the epilimnion and metalimnion using an integrating sampler (Shearer 1978). A 125-mL subsample was preserved in acid Lugol's solution. A 2-mL aliquot was extracted and gravity settled for 24 h. Species identification and enumeration were performed on an inverted microscope at 125X and 550X using the Ütermohl technique (Nauwerck 1963). Cell measurements were performed on each species to a maximum of 50 individuals. Cell numbers were then converted to wet weight biomass by estimating cell volumes of individual species from geometric formulae best fitted to their shapes, and assuming a specific gravity of 1.0 (Vollenweider 1968). Heterocysts were enumerated and measured separately from vegetative cells.

N_2-Fixation Measurements

N_2-fixation in Lake 227 was measured in June, July, and August of 1988 and 1989 and also in 1990–1992 after the cessation of artificial N addition. From 1988 to 1991, these measurements were not made on the same samples as taken for phytolankton analysis; in 1992, both measurements were performed on the same samples. The N_2-fixation results of 1989 have not been included here, however, because of differences between years in both sampling and analytical protocols (Hendzel et al. 1994).

TABLE 2. Annual total inputs, observed changes in lake mass, ratios, and residence times for nitrogen (N) and phosphorus (P) in Lake 227 from 1970 to 1992.

Year	N inputs (kg)	N losses outflow (kg)	N changes in lake mass (kg)	N losses sediment (kg)	N lake mass (kg)	P inputs (kg)	P losses outflow (kg)	P changes in lake mass (kg)	P losses sediment (kg)	P lake mass (kg)	Lake mass N:P ratio (kg/kg)	Residence (yr)	
												N	P
1970	350	114	112	162	205	26	2.85	2.82	20.78	6	33	0.59	0.23
1971	374	75	10	128	179	28	4.46	2.82	21.12	9	21	0.48	0.30
1972	369	70	387	140	239	26	3.59	−1.27	23.32	8	31	0.65	0.30
1973	355	67	17	144	259	26	3.72	1.61	20.44	9	28	0.73	0.35
1974	363	68	43	177	302	26	1.86	−0.62	24.59	8	38	0.83	0.31
1975	167	76	25	168	304	24	2.57	−1.21	22.67	6	48	1.82	0.26
1976	160	39	−49	200	279	24	0.81	−0.47	23.42	7	38	1.74	0.31
1977	183	49	31	137	311	25	2.14	3.47	18.89	12	27	1.70	0.48
1978	169	69	65	92	383	24	2.76	4.12	17.19	14	27	2.27	0.58
1979	200	62	39	152	400	24	2.01	−0.43	22.61	13	31	2.00	0.54
1980	191	31	35	146	450	24	0.71	2.66	20.38	18	25	2.35	0.76
1981	186	56	−57	161	353	24	2.45	−1.67	23.26	13	27	1.90	0.54
1982	253	91	34	186	471	25	2.79	−3.75	25.40	13	38	1.86	0.51
1983	182	33	119	86	515	24	1.46	5.98	16.57	17	31	2.83	0.69
1984	202	20	83	171	425	24	1.12	−2.08	25.03	14	30	2.11	0.60
1985	234	122	−37	198	416	26	4.03	−4.80	26.33	10	43	1.78	0.38
1986	197	53	−9	176	402	25	1.24	−0.59	23.63	10	40	2.05	0.41
1987	185	22	21	163	433	24	0.77	3.25	19.48	13	31	2.34	0.57
1988	208	33	9	161	438	25	0.96	1.58	21.81	15	29	2.11	0.61
1989	218	44	4	113	457	25	2.79	1.48	20.16	16	29	2.10	0.65
1990	87	26	−1	135	427	24	1.95	−2.66	24.62	13	32	4.91	0.56
1991	104	70	−43	153	395	24	2.23	−2.14	24.13	11	36	3.82	0.46
1992	161	123	−16	154	379	26	3.53	−1.43	23.20	10	39	2.35	0.38

N_2-fixation was measured using flame ionization detector gas chromatography, applying a modification (Hendzel et al. 1994) of the glass syringe acetylene reduction method of Flett et al. (1976). In 1988, 1990, and 1991 a 30-mL aliquot of a surface sample was placed in a 50-cm^3 syringe, injected with 10 cm^3 of acetylene, and incubated at a constant light level for 4 h. In 1992, N_2-fixation was measured from integrated epilimnetic and metalimnetic samples using a similar protocol. A subsample from each integrated sample in 1992 was incubated at three decreasing light levels and the relationship of N_2-fixation to light availability was determined (Hendzel et al. 1994). To estimate the daily integral rate of N_2-fixation over the growing season, measured samples were time weighted.

Light Measurements

Daily fluxes of photosynthetically available radiation (PAR) ($\mu E \cdot m^{-2} \cdot d^{-1}$) were monitored over each ice-free season at 10 min intervals using a LI-COR data logger (Fee et al. 1992).

Chemical Analysis

Lake water samples for chemical analysis were collected simultaneously with the phytoplankton samples. Lake water chemistry was characterized using monthly depth profiles of the entire water column, and weekly to biweekly sampling of the epilimnion. The Lake 227 outflow stream was also sampled when it was flowing. Bulk precipitation was sampled on an event basis using a continuously open collector (Linsey et al. 1987). The northwest inflow to Lake 239 was used to estimate direct runoff water and nutrient inputs to Lake 227 and was sampled either weekly or on an event basis during spring runoff or major storms. Dissolved and particulate C, N, and P and dissolved sodium (Na) were measured using analytical methods described in Stainton et al. (1977). Total N (TN) and total P (TP) were obtained by summing the dissolved and particulate fractions.

N and P Sedimentation

Other than sedimentation, there is no significant loss pathway for P from Lake 227; we assumed that the N:P ratio in material sedimenting from the water column was that of the seston. Sedimentary losses of N and P were estimated using the N:P ratios in layers of known age from a Lake 227 sediment core (Wolfe et al. 1994). Because the core only included inputs up to 1985, sedimentation losses of N and P from 1986–1992 were assumed to be similar to those measured for 1980–1985. Direct sedimentation for Lake 227 was measured in 1992 using sediment traps suspended at 6 m (J. Elser, Arizona State University, Tempe, Ariz., unpublished data); this measure supported the assumptions made for P sedimentation and the estimates of N sedimentation based on seston composition and P retention (Fig. 1).

Chemical Budgets

Annual total inputs of TN and TP to Lake 227 were calculated as the sums of direct precipitation inputs to the lake surface, direct watershed runoff (there is no input stream), chemical fertilizer additions, and N_2-fixation (Tables 1 and 2). Direct runoff estimates for water volume and nutrient inputs were determined by extrapolation from a hydrological index basin, the northwestern basin of Lake 239 (Schindler et al. 1987). Chemical additions were reported by Cruikshank (1984a, 1986, 1991).

Annual losses on TN and TP were calculated as the sum of outflow and sedimentation losses (Table 2). Outflow losses were estimated from continuous stream flow records (K. Beaty, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data) and from weekly chemical analysis of stream and epilimnion samples. Estimates of annual N and P sedimentation were derived from the P budget and the N:P ratio of seston as described above.

Annual inputs and losses were used to derive an estimate of the annual change in the mass of N and P in the lake (Table 2). The instantaneous mass of elements in Lake 227 was calculated using the vertical profiles of epilimnetic chemistry described above, lake bathymetry (Brunskill and Schindler 1971), thermocline depth (Cruikshank 1984b), lake stage, and ice thickness (Beaty 1981, 1984). Observed mean annual lake mass was the time-weighted average of the monthly masses.

The difference between the observed change in whole lake mass and the calculated change in whole lake mass from inputs and outputs on an annual basis were used to estimate unmeasured sources (a positive difference) or sinks (a negative difference) of N and P. These annual differences also include all error terms. Residence times for TN and TP in the water column were calculated using the annual mean lake mass and total annual inputs (Janus and Vollenweider 1981).

Results

From 1970 to 1974, Lake 227 was fertilized with 315 kg N and 24 kg P (N:P ratio 13:1, Table 1) (Schindler 1974). These additions caused an immediate increase in phytoplankton biomass above prefertilization levels, and algal dominance shifted from chrysophytes, which are characteristic of oligotrophic lakes of the region, to a codominance by small chlorophytes (generally unicellular desmids) and *Oscillatoria redekei* Van Goor, a filamentous nonheterocystous cyanobacterium (Fig. 2; Schindler et al. 1971, 1973; Findlay and Kling 1975).

When the N:P loading ratio of Lake 227 was lowered to 5:1 in 1975, total phytoplankton biomass remained elevated but *Aphanizomenon gracile*, a heterocystous cyanobacterium, codominated the assemblage during late summer (Figs. 2 and 3b). Its appearance and increased abundance followed a seasonal succession of *Scenedesmus brevispina* (G.M. Smith) Chodat, *S. denticulatus* Lagerheim, *Oocystis lacustris* (Chodat), *O. submarina* var. *variabilis*, and *Dictyosphaerium simplex* Skuja, small chlorophytes, and the filamentous cyanobacterium *Oscillatoria redekei* (Findlay 1978). This seasonal succession from small chlorophytes and *Oscillatoria* to dominance by *A. gracile* continued in Lake 227 until 1977 (Findlay 1981), when *Aphanizomenon schindleri* Kling, Findlay, Komarek (Kling et al. 1994) and *A. gracile* codominated the assemblage (Fig. 3b).

From 1978 to 1983, *A. schindleri* dominated the phytoplankton assemblages during midsummer, and total biomass exceeded 35 $g \cdot m^{-3}$ (Figs. 2 and 3b) (Findlay 1983). However, from 1983 to 1985, and again in 1988, *A. schindleri* occurred in lower abundances and dominated the late summer phytoplankton assemblages <40% of the time (Figs. 2 and 3b). During these years of reduced *A. schindleri* dominance, small chlorophytes (*Spondylosium planum* G.S. West,

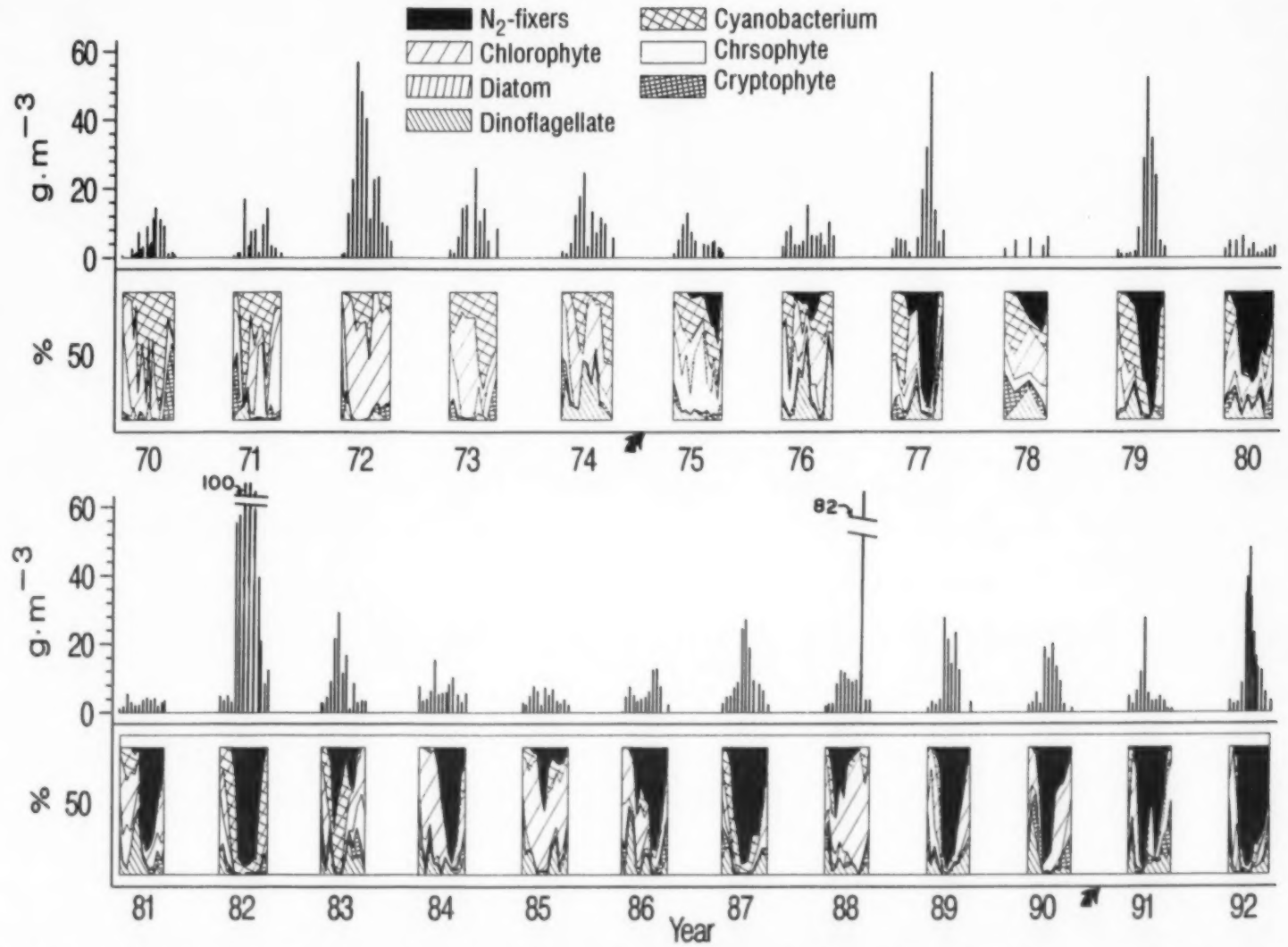

FIG. 2. Daily ice-free season epilimnetic phytoplankton biomass and accumulative percent composition for Lake 227 from 1970 to 1992. Arrows indicate changes in the fertilizer addition nitrogen (N): phosphorus (P) ratios (by weight) in 1975 (from 13:1 to 5:1) and in 1990 (from 5:1 to P only).

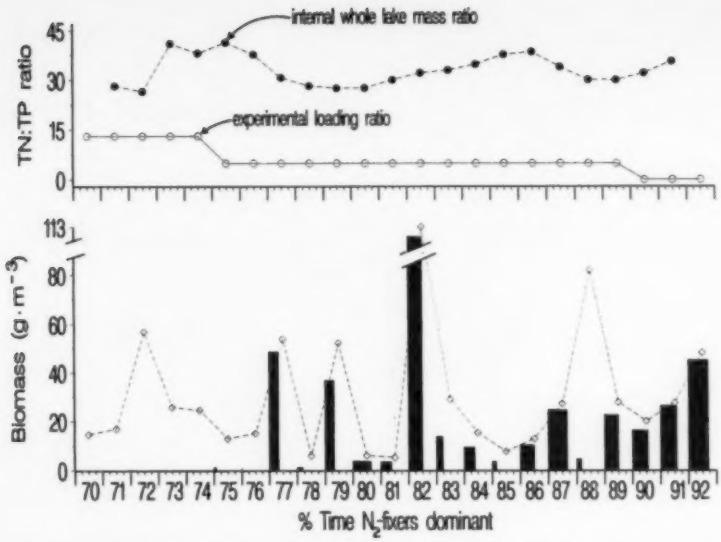

FIG. 3. (a) Whole lake total nitrogen (TN) : total phosphorus (TP) ratios (based on whole lake internal masses, Table 2) and external loading TN:TP ratio (fertilizer additions) for Lake 227 from 1970 to 1992. Data points for whole lake mass ratio are calculated 3-yr running means. (b) Intensity and duration of N_2-fixing cyanobacterial blooms (solid bars) and maximum seasonal biomass (open diamonds) in the epilimnion of Lake 227 from 1970 to 1992. Intensity of N_2 fixers is the maximum biomass of N_2-fixing species occurring in a season, and duration is the percentage of days in a season (in 25% increments) dominated by N_2 fixers representing >30% of the total daily biomass.

Scenedesmus denticulatus, *S. brevispina*, *Oocystis lacustris*, and *Dictyosphaerium simplex*) and coccoidal cyanobacteria (*Synechococcus* sp.) dominated the phytoplankton community throughout June and early July. *Oscillatoria redekei* was also prominent in the community throughout this period, but this species was much decreased in overall abundance compared with the early years of fertilization.

In 1987 and 1989, *A. schindleri* again dominated the phytoplankton assemblage for much of the open-water season (Fig. 3b), and achieved higher peak biomasses than in 1983–1986 (Fig. 2). *Synechococcus* sp. remained abundant but contributed little to overall biomass because of its small size.

In 1990, epilimnetic additions of N ceased, and P additions continued (Table 1). *Synechococcus* sp. then significantly decreased, and *A. schindleri* dominated the algae for much of the 1990–1992 open-water seasons; total biomass once again exceeded 35 $g \cdot m^{-3}$ by 1992 (Figs. 2 and 3b).

In all years, heterocyst abundance began increasing in mid-June and accelerated to a maximum by mid-July to mid-August (Fig. 4), declining sharply thereafter. On occasion, a second peak of heterocyst abundance occurred in early September, but it was never of the same magnitude or duration as the summer peaks (Fig. 4). Heterocyst abundance in Lake 227 was significantly correlated with *Aphanizomenon* vegetative cell abundances on all sampling dates (Fig. 5).

During 1992, N_2-fixation measurements and heterocyst enumerations were performed on subsamples from the same samples. This allowed calculation of N_2-fixation rates per heterocyst. Integrated epilimnetic and metalimnetic samples incubated at three different light intensities indicated that N_2-fixation was saturated at very low light levels and was relatively constant throughout the epilimnion (Hendzel et al. 1994) (Fig. 6). Ninety percent of the fixation occurred in the epilimnion. Specific fixation rates (N_2 fixed per heterocyst) were substantially higher in the epilimnion, although fixation was proportional to heterocyst numbers over the season in both depth strata. Epilimnetic N_2-fixation became detectable in mid-June and reached a maximum rate by the end of June, whereas maximum heterocyst abundance occurred in mid-July (Fig. 6). A second, much smaller peak of fixation occurred in September, coinciding with a second peak in heterocyst abundance.

The relationship of the rate of N_2-fixation ($\mu g \cdot L^{-1} \cdot h^{-1}$) to heterocyst abundance ($cells \cdot L^{-1}$) during the entire 1992 season was highly significant and explained 64% of the variance in epilimnetic fixation (Fig. 7a). Separating the data into summer (May–August) and fall (September–October) and comparing the relationship for the two seasons through a linear regression indicates that the slopes and intercepts are not significantly different ($P = 0.94$ and 0.626, respectively).

Fall peaks, such as occurred in 1992, were infrequent in Lake 227 (perhaps because of decreasing day length in late summer) and may be a response to entrainment of P-rich water from the metalimnion during autumnal mixing, or perhaps to entrainment of N_2-fixers persisting in this layer. The variance accounted for in the specific N_2-fixation rate increased ($r^2 = 0.90$, $P = 0.0011$, $n = 7$) when the fall peak was eliminated and heterocyst abundance was regressed against fixation on the previous sampling date (Fig. 7b). In 1988, 1990, and 1991, N_2-fixation was measured sporadically (n = 3–7) and usually 4–7 d in advance of sampling for phytoplankton community analysis. However, the data reveal a pattern of maximum fixation occurring in advance of maximum heterocyst abundance, similar to that documented in 1992 (Fig. 4).

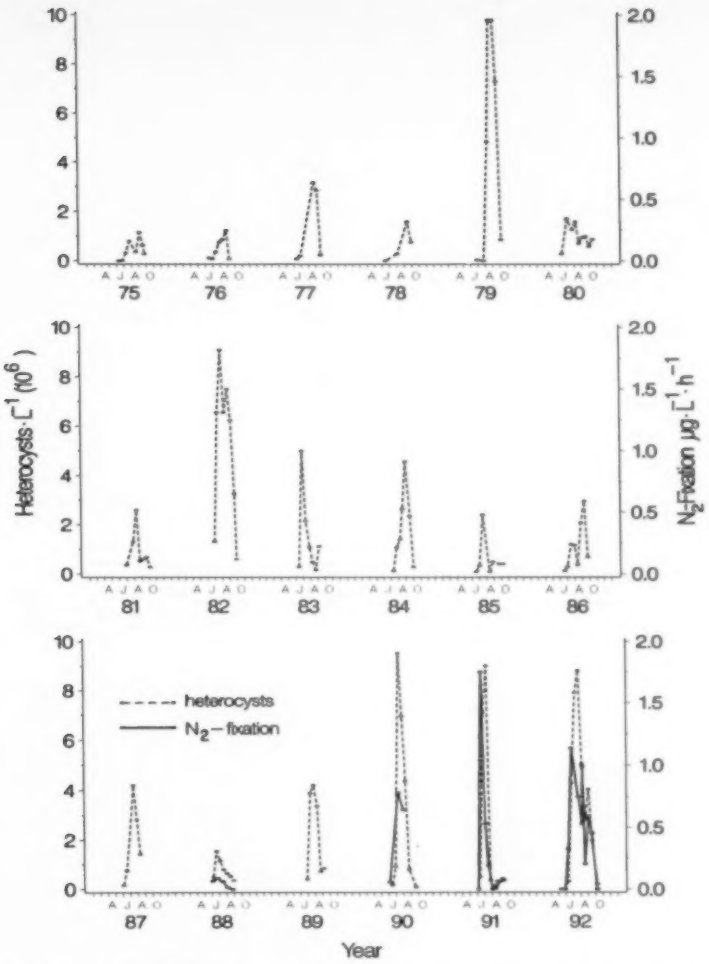

FIG. 4. Daily heterocyst abundance (cells · L^{-1}) and measured rates of N_2-fixation (µg · L^{-1} · h^{-1}) for Lake 227 from 1975 to 1992.

Adjustment of sampling dates in 1988, 1990, and 1991, using a 2-wk lag interval, the period suggested from the 1992 data, strengthened the relationship between fixation and heterocyst abundance ($r^2 = 0.79$, $P = 0.0001$). Based on 1988, 1990, 1991, and 1992, a significant relationship does exist between mean annual fixation and mean annual heterocyst abundance (Fig. 7b). A comparison between the 1992 summer relationship and the mean annual relation indicates that the slopes and intercepts are not significantly different ($P = 0.92$ and 0.52, respectively) and, therefore, a common predictive relationship is assumed to exist (Fig. 7b). The fact that the 1992 annual mean estimate, including both summer and fall peaks, is very close to the predicted line (based on annual means) suggests that the fall peak contributes little to annual fixation. The 1992 seasonal relationship, excluding the fall samples and regressing heterocyst abundance against fixation on the previous sampling date, is highly significant and has a low variance; hence, the daily integral rate of N_2-fixation (µg·L^{-1}·d^{-1}) during periods when heterocysts were present was calculated for past years using the linear regression equation:

$$(1)\quad N_{2p} = 1.25 \times 10^{-7}\,(\text{heterocyst abundance}) - 0.0097$$

where N_{2p} is the predicted daily rate of N_2-fixation. Hendzel et al. (1994) documented fixation occurring at low saturation light levels. Therefore, we chose a mean daily saturation light level consistent with that observed by Hendzel et al. (1994) for estimating the hours of available daylight for fixation. Total N inputs (kg) from N_2-fixation from 1975–1992 were calculated using

$$(2)\quad TN_{2p} = \Sigma\,[N_{2p}\,(\text{strata volume})\text{day length}] \times 10^6$$

where strata volume = daily volume of the mixed layer, and day length = measured hours of light (above saturation light levels required for fixation) available for fixation.

Inferred N inputs via fixation accounted for as much as 77 kg in 1982 and up to 53% of the total annual N inputs in 1990 (Table 1). Compared with actual measured fixation estimated for 1988, 1990, 1991, and 1992 by Hendzel et al. (1994), the inferred estimates tend to be somewhat conservative (Table 1).

Budget calculations for the Na balance indicate that Na behaves conservatively (Fig. 8b). In general, the calculated

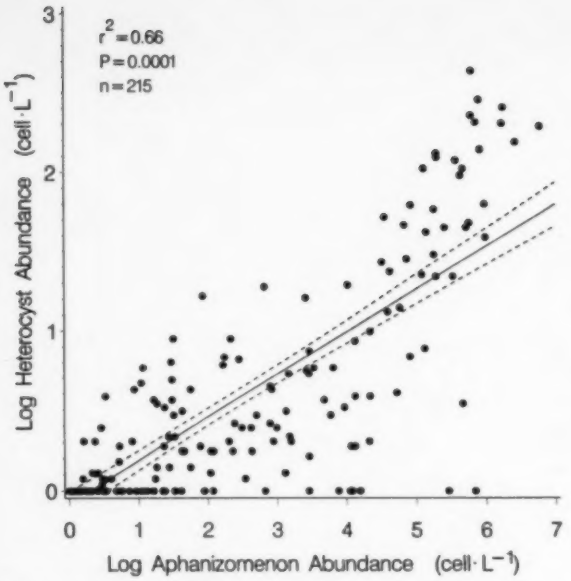

FIG. 5. Linear regression of heterocyst abundance (cells · L^{-1}) and *Aphanizomenon* abundance (cells · L^{-1}) in Lake 227 for all sampling dates from 1975 to 1992. The dashed lines represent the 95% confidence interval for the mean predicted values.

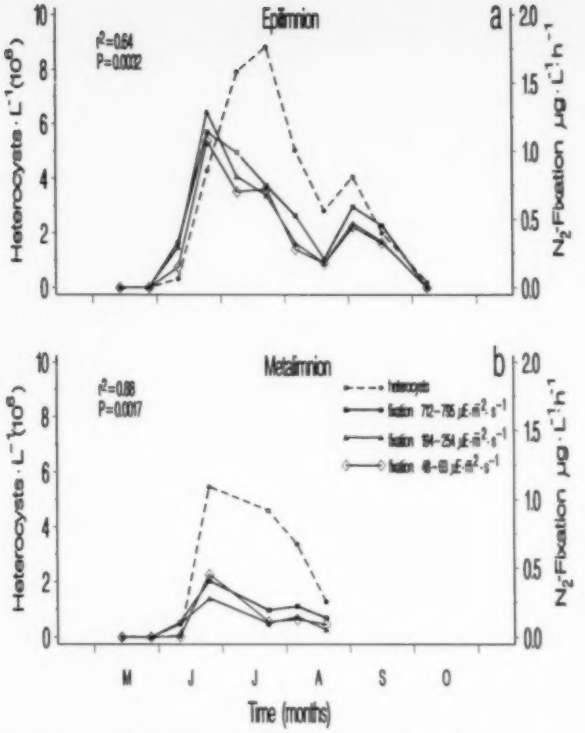

FIG. 6. (a) Epilimnetic and (b) metalimnetic daily heterocyst abundance (cells · L^{-1}) and rate of N_2-fixation (µg · L^{-1} · h^{-1}) at three decreasing light levels for Lake 227 from May to October 1992. The r^2-values are the amounts of variance in fixation (at the highest light level) explained by regressing N_2-fixation on heterocyst abundance (data are not lagged), and *P* is the associated probability.

N budgets, with fixation and sedimentation, balance as well as the Na budget, although N is not conservative. This increases our confidence in the correctness of the N budget (Table 2). Detailed examination of the historic N budgets indicate a period of an unaccounted N sink (1971–1974) followed by a period of an unaccounted N source (1975–1991) (Fig. 8b). The period 1970–1974 involved large weekly pulse additions of NO_3 to the epilimnion; lake chemistry was analyzed 6 d later. NO_3 concentrations at times of addition could have reached levels as high as 372 µg·L^{-1}. This would have resulted in periods (days) of high NO_3 concentrations that could have stimulated denitrification. Because daily NO_3 levels were not monitored, it is not possible to quantitatively estimate denitrification (Kelly et al. 1982), but the unmeasured denitrification would appear in the budget as an unaccounted N sink. The unaccounted N source from 1975 to 1992 may result from our conservative estimate of planktonic N_2-fixation as stated above and/or from unmeasured benthic fixation.

Discussion

Regulation of N_2-fixation

The observed relationship between rates of N_2-fixation and heterocyst abundance in Lake 227 from 1989 to 1992 allowed us to estimate atmospheric N_2-fixation inputs from historical phytoplankton data. The fixation estimates derived from the relationship appear conservative compared with the N_2-fixation estimates for Lake 227 in 1975 (Flett et al. 1980), and in 1988, 1990, 1991, and 1992 (Hendzel et al. 1994). However, estimates of Flett et al. (1980) and Hendzel et al. (1994) depend on only a few high measurements made near the peak of the blooms, and extrapolated to the entire season; these estimates are possibly biased high. The 1992 estimates of N inputs by fixation, performed on the same samples as those analyzed for phytoplankton, and those predicted from the regression equation differed by ~10%. Based on the conservative predicted values, heterocystous cyanobacteria in Lake 227 at times contributed significant amounts of N (4–53% of the total input) to the system (Table 1); they contributed perhaps even more based on in situ measurements. Nitrogen inputs via fixation have been documented to represent as much as 10–80% of the N inputs into a variety of lakes (Levine and Lewis 1987; Rönicke 1986; Howarth et al. 1988a, 1988b).

Schindler et al. (1987) presented a N budget and balance for Lake 227 (1969–1982) without accounting for fixation. From 1977 to 1982, they documented an unknown N source, which they speculated was fixation by heterocystous cyanobacteria. Inclusion of the N inputs by fixation into the Lake 227 budget from 1970 to 1992 largely eliminated this discrepancy, with the exception of the period 1970–1974 when there appears to have been an unaccounted sink (Fig. 8b). From 1969 to 1974, Lake 227 was artificially fertilized with $NaNO_3$ at an N:P ratio of 13:1. Elevated NO_3 concentrations were documented over this period, and we believe that this stimulated denitrification, thus accounting for the loss. Chan and Campbell (1980) measured denitrification in Lake 227 (1973–1975) and concluded that nearly

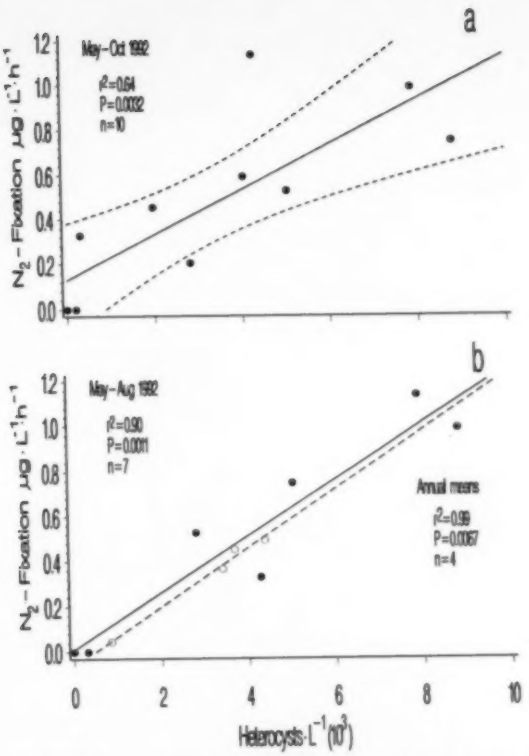

FIG. 7. (a) Linear regression of heterocyst abundance and the N_2-fixation rate in Lake 227 from May to October 1992. The dashed lines are the 95% confidence intervals. (b) The N_2-fixation data lagged by 2 wk. The solid line represents the predicted values. The four open circles are the measured annual means for 1988, 1990, 1991, and 1992. The dotted line represents the predicted annual values from the regression.

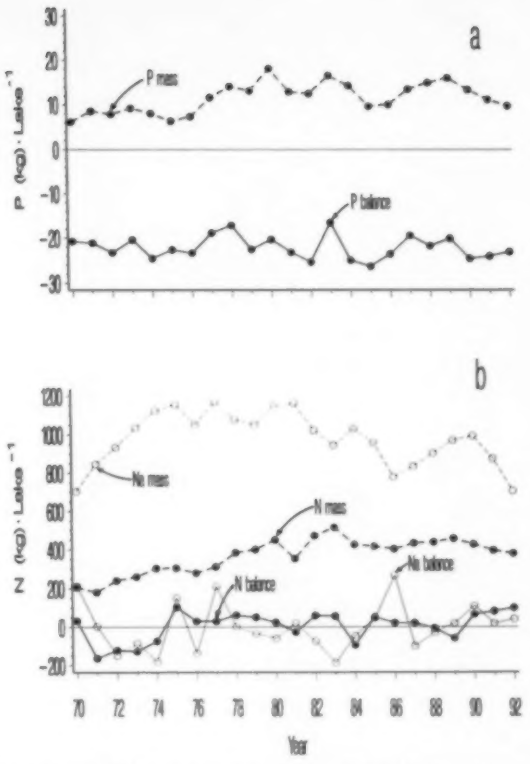

FIG. 8. Annual internal mass and balance for (a) phosphorus (P) and (b) nitrogen (N) and sodium (Na) in Lake 227 from 1970 to 1992.

all the NO_3 that mixed into the surficial sediments was denitrified. They estimated that denitrification removed 1.4% of the annual epilimnetic input, which is a small proportion of that unaccounted for in the N balance (Fig. 8b). The changed method of addition and reduced loading rates in 1975 would have eliminated the high pulses of NO_3 concentration and thereby reduced denitrification losses.

Regulation of Cyanobacterial Dominance

There are currently several hypotheses to explain cyanobacterial dominance in freshwater lakes. Shapiro (1990) reviewed many of these, dismissing hypotheses based on low light, elevated water temperatures, changes in N:P ratio, buoyancy regulation, and zooplankton grazing; he concluded that low CO_2 and high pH are the most important factors. Our 23-yr historical record for Lake 227 also provides little support for hypotheses based on low light and high temperature. From 1970 to 1992, mean irradiance in the water column in Lake 227 has remained relatively constant, varying less than 20% annually (J. Shearer, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data). Yet, from 1970 to 1974 chlorophytes dominated the phytoplankton, although nonheterocystous cyanobacteria increased, and from 1975 to the present, heterocystous cyanobacteria dominated. Even though 1992 was the coolest year on record (K. Beaty, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data), it had the second highest yield of cyanobacteria of the historical record. A series of mesocosm experiments revealed that buoyancy regulation is not a factor in cyanobacterial dominance in Lake 227 (A. Klemer, University of Minnesota, Duluth, Minn., unpublished data). Malley et al. (1988) described a dramatic decrease in crustacean zooplankton, which decreased grazing pressure in Lake 227; they hypothesized that the decrease was caused by elevated pH. From 1970 to 1992, pH in Lake 227 increased seasonally from ~6.7 in spring to midsummer values of >9.0. Cyanobacteria dominated in many years (Fig. 3b), but in 1972, 1973, 1984, 1985, and 1988 chlorophytes were continuously present and usually dominated the midsummer phytoplankon assemblage, even though pH exceeded 9 and DIC was low (<40 $\mu g \cdot L^{-1}$). Thus, the documented responses of the phytoplankton community in Lake 227 do not support Shapiro's (1990) hypothesis that low CO_2 and high pH necessarily ensure cyanobacterial dominance in freshwater lakes.

Blooms of N_2-fixing *Aphanizomenon* in Lake 227 have occurred annually since the N:P fertilizer input ratio was decreased to 5:1 (Figs. 2 and 3b). Flett et al. (1980) hypothesized that N:P external loading ratios <10:1 by mass dictated the occurrence of these blooms, and the results from Lake 227 up to the time of Flett et al.'s study support this hypothesis. However, the intensity of these blooms has been

unpredictable (Fig. 3b) despite a constant external experimental loading ratio in Lake 227 from 1975 to 1989.

We favour the hypothesis that N:P loading ratios determine whether blooms of heterocystous cyanobacteria occur (Toetz and McFarland 1987; Smith 1990). Total N in Lake 227 has increased over time (Table 2, Fig. 8b), caused first by heavy N loading and retention, and then by N loading supplemented by fixation. Because fixation outstripped denitrification and N losses to sedimentation and outflow over time (Schindler et al. 1987), the total N mass and concentration in Lake 227 increased until the mid-1980s when a new steady state was apparently attained (i.e., losses of N to denitrification, sedimentation and outflow balanced inputs). Total P showed a similar increase over time (Fig. 8a), but rather than the gradual rise that total N exhibited there was a sharper increase through the late 1970s. Because of these increases in TN and TP there is little evidence for a net change in the TN:TP mass ratio in Lake 227 over time (Fig. 3a), despite substantial changes in the experimental loading of N. The mean lake mass N:P ratio is 31:1; annual extremes reach 21:1 and 48:1 (Table 2). In 1970, the second year of 13:1 experimental loading, the ratio was 33:1; in 1992, after 3 yr of 0:1 experimental loading, the ratio was 39:1. Given that the residence time of the TN pool in the lake has varied from 0.5 yr in 1971 to 4.9 yr in 1990 and the TP pool has varied from 0.23 yr in 1970 to 0.76 yr in 1980, the relative constancy of the lake mass TN:TP ratio is surprising. The relative constancy of the TN:TP ratio over 23 yr seems dependent on the constant experimental P loading; N_2-fixation adds N or denitrification eliminates N, depending on the experimental N loading. Mesocosm experiments by Levine and Schindler (1992) and R. Hecky (Department of Fisheries and Oceans, Winnipeg, Man., unpubl. data), have also demonstrated the importance of N:P loading ratios in influencing cyanobacteria blooms.

If the N:P ratio of the inorganic nutrients available to the algae from regeneration is assumed to be a relatively constant fraction of the internal TN and TP mass, then the internal loading ratio of N:P has changed little over the 23-yr record. Despite the tendency of long-term constancy, there is substantial annual variability (range 21–48) of the internal TN:TP ratio (Table 2). When annual values are smoothed by taking a 3-yr running mean, then a cyclical pattern is suggested, and high TN:TP periods are associated with less intense bloom conditions (Fig. 3). The change in the external loading ratio in 1990 again reduced the N:P supply ratio available to the algae, and led to strong heterocystous cyanobacterial blooms in three successive years. However, unlike Flett et al. (1980), we believe it is the N:P ratio of available inorganic nutrients exposed to the cell that is important (i.e., the net result of the external and internal loading of inorganic N and P). We forecast that the intensity and occurrence of cyanobacteria blooms will be more predictable without experimental loading of N because, without N experimental loading, high annual rates of N_2-fixation will be necessary to offset N losses to sedimentation and denitrification, and heterocystous cyanobacteria will dominate every year. The long-term stability of the internal TN:TP ratio is consistent with such a biogeochemical compensation.

The long-term history of algal blooms in Lake 227 explains why efforts to predict cyanobacterial blooms from only external loading ratios of N and P or in-lake TN:TP concentrations have frequently failed (Toetz and McFarland 1987; Lukarelich and McComb 1986). Both external and internal nutrient sources must be evaluated because both sources are relevant to the phytoplankton (Levine and Schindler 1992; Smith 1990). At high N:P external loading ratios in Lake 227, heterocystous cyanobacteria did not bloom (Fig. 3). At intermediate experimental ratios (5:1), blooms occurred. However, because the internal regeneration of nutrients occurs at a high N:P ratio in Lake 227 (Hendzel et al. 1994), bloom occurrence, intensity, and duration were unpredictable (Fig. 3), depending on the strength of the internal cycle. At extremely low N:P external loading ratios (1990 and after), blooms of heterocystous cyanobacteria were required every year to meet the N demand, and so blooms became predictable. The balancing of N inputs and losses for any given P load requires that water reside in the lake long enough for internal processes to come to steady state with P loading (Schindler et al. 1987). Thus, the ratio of N_2-fixation to N loss processes will determine whether systems are N sufficient or N deficient. For example, tropical lakes with long water residence times are probably prone to high rates of denitrification in their warm, hypoxic metalimnion, and thus they remain chronically N deficient (Hecky 1993). In such systems, which have a high continuous loss of gaseous N_2 and nitrous oxide (N_2O) (by denitrification), N_2-fixation is required to continuously compensate for these losses. Total N demand will be set by P loading, and eutrophication of tropical systems will rapidly lead to increased dominance by heterocystous cyanobacteria (Hecky 1993). As noted by Levine and Schindler (1992), both N_2-fixation and denitrification deserve much further research and modelling in order to improve our ability to predict cyanobacterial blooms.

Phosphorus supply can drive both N_2-fixation (at low N:P supply ratio) and denitrification (through its effect on the oxygen balance of eutrophied systems) but the set point of steady-state N:P ratio in the water column will be system specific. However, there is no doubt from the historic experimental challenges to the Lake 227 ecosystem that the N:P ratio of external and internal sources has determined the occurrence of heterocystous cyanobacterial blooms.

Conclusions

Heterocyst abundance can be used to infer N_2-fixation rates. N_2-fixation by heterocystous cyanobacteria is a major (previously unmeasured) component of the historic N budget of Lake 227. The Lake 227 phytoplankton results support the hypothesis that N:P loading ratios influence cyanobacterial blooms. Based on the 23-yr record for Lake 227, we conclude that heterocystous cyanobacterial dominance becomes increasingly predictable as external loading ratios of N:P decline to low values (below 5:1 in Lake 227). Predictability at intermediate ratios is low; the external load is offset by an internal load of high N:P in Lake 227 because N is more efficiently recycled than P. Interannual variation in the relative strength of these loading sources at intermediate external N:P ratios (e.g., 5:1) creates uncertainty, although the long-term mean condition clearly favours heterocystous cyanobacteria over other algal groups. Lowering the experimental loading ratio of N:P in 1975 clearly shifted the community from chlorophycean dominance towards heterocystous cyanobacteria, but with substantial interannual

variability. An extreme reduction of the external loading ratio in 1990 subsequently increased the predictability of the heterocystous cyanobacterial blooms. The internal TN:TP ratio changed little over time and, therefore, is a less useful predictor per se of cyanobacterial dominance than the external loading ratio.

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References

BEATY, K.G. 1981. Hydrometeorological data for the Experimental Lakes Area, northwestern Ontario, 1969 through 1978. Can. Data Rep. Fish. Aquat. Sci. 285: 317 p.

BEATY, K.G. 1984. Hydrometeorological data for the Experimental Lakes Area, northwestern Ontario, 1979–1981. Can. Data Rep. Fish. Aquat. Sci. 480: 146 p.

BRUNSKILL, G.J., AND D.W. SCHINDLER. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139–155.

CHAN, Y.K., AND N.E.R. CAMPBELL. 1980. Denitrification in Lake 227 during summer stratification. Can. J. Fish. Aquat. Sci. 37: 494–505.

CRUIKSHANK, D.R. 1984a. Whole lake chemical additions in the Experimental Lakes Area, 1969–1983. Can. Fish. Mar. Serv. Data Rep. 449: 23 p.

CRUIKSHANK, D.R. 1984b. The relationship of summer thermocline depth to several physical characteristics of lakes. Can. Fish. Mar. Serv. Data Rep. 1245: 33 p.

CRUIKSHANK, D.R. 1986. Whole lake chemical additions in the Experimental Lakes Area, 1984–1985. Can. Data Rep. Fish. Aquat. Sci. 580: 10 p.

CRUIKSHANK, D.R. 1991. Whole lake chemical additions in the Experimental Lakes Area, 1986–1989. Can. Data Rep. Fish. Aquat. Sci. 816: 18 p.

FEE, E.J., J.A. SHEARER, E.R. DEBRUYN, AND E.U. SCHINDLER. 1992. Effects of lake size on phytoplankton photosynthesis. Can. J. Fish Aquat. Sci. 49: 2445–2459.

FINDLAY, D.L. 1978. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1974–1976. Can. Fish. Mar. Serv. MS Rep. 1466: 41 p.

FINDLAY, D.L. 1981. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1977–1979. Can. MS Rep. Fish. Aquat. Sci. 1627: 40 p.

FINDLAY, D.L. 1983. Seasonal successions of phytoplankton in five lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1980–1982. Can. MS Rep. Fish. Aquat. Sci. 1710: 30 p.

FINDLAY, D.L., AND H.J. KLING. 1975. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lake Area, northwestern Ontario, following artificial eutrophication. Can. Fish. Mar. Serv. Tech. Rep. 513: 53 p.

FLETT, R.J., R.D. HAMILTON, AND N.E.R. CAMPBELL. 1976. Aquatic acetylene-reduction techniques: solutions to several problems. Can. J. Microbiol. 22: 43–51.

FLETT, R.J., D.W. SCHINDLER, R.D. HAMILTON, AND N.E.R. CAMPBELL. 1980. Nitrogen fixation in Canadian Precambrian Shield lakes. Can. J. Fish. Aquat Sci. 37: 494–505.

HANEY, J.F. 1987. Field studies on zooplankton–cyanobacteria interactions. N.Z. J. Mar. Freshwater Res. 21: 467–475.

HECKY, R.E. 1993. Eutrophication of Lake Victoria. Verh. Int. Ver. Theor. Agnew. Limnol. 25: 39–48.

HENDZEL, L.L. R.E. HECKY, AND D.L. FINDLAY. 1994. Recent changes of N_2-fixation in Lake 227 in response to reduction of the N:P loading ratio. Can. J. Fish. Aquat. Sci. 51: 2247–2253.

HOLMGREN, S. 1983. Phytoplankton biomass and algal composition in natural, fertilized and polluted subarctic lakes. Acta Univ. Upps. Abstr. Uppsa. Diss. Fac. Sci. 674: 125 p.

HOWARTH, R.W., R. MARINO, J. LANE, AND J.J. COLE. 1988a. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. Limnol. Oceanogr. 33: 669–687.

HOWARTH, R.W., R. MARINO, AND J.J. COLE. 1988b. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. Limnol. Oceanogr. 33: 688–701.

JANUS, L.L., AND R.A. VOLLENWEIDER. 1981. The OECD cooperative programme on eutrophication. Canadian contribution. Can. Inland Waters Dir. Sci. Ser. 131: 381 p.

KELLY, C.A., J.W.M. RUDD, R.B. COOK, AND D.W. SCHINDLER. 1982. The potential importance of bacterial processes in regulating rate of lake acidification. Limnol. Oceanogr. 27: 868–882.

KING, D.L. 1970. The role of carbon in eutrophication. J. Water Pollut. Control Fed. 42: 2035–2051.

KLEMER, A.R., J. FEUILLADE, AND M. FEUILLADE. 1982. Cyano-bacterial blooms: carbon and nitrogen limitation have opposite effects on the buoyancy of Oscillatoria. Science (Wash., D.C.) 215: 1629–1631.

KLING, H.J., D.L. FINDLAY, AND J. KOMÁREK. 1994. *Aphanizomenon schindleri* sp.nov.: a new nostocacean cyanoprokaryote from the Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 51: 2267–2273.

LEVINE, S.N., AND W.M. LEWIS. 1987. A numerical model of nitrogen fixation and its application to Lake Valencia, Venezuela. Freshwater Biol. 17: 265–274.

LEVINE, S.N., AND D.W. SCHINDLER. 1992. Modification of the N:P ratio in lakes by *in situ* processes. Limnol. Oceanogr. 37: 917–935.

LINSEY, G.A., D.W. SCHINDLER, AND M.P. STAINTON. 1987. Atmospheric deposition of nutrients and major ions at the Experimental Lakes Area in northwestern Ontario, 1970–1982. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 206–214.

LUKATELICH, R.J., AND A.J. MCCOMB. 1986. Nutrient levels and the development of diatom and blue–green algal blooms in a shallow Australian estuary. J. Plankton. Res. 8: 597–618.

MALLEY, D.F., P.S.S. CHANG, D.L. FINDLAY, AND G.A. LINSEY. 1988. Extreme perturbation of the zooplankton community of a small Precambrian Shield lake by the additions of nutrients. Verh. Int. Ver. Theor. Angew. Limnol. 23: 2237–2247.

MCQUEEN, D.J., AND D.R.S. LEAN. 1987. Influence of water temperature and nitrogen to phosphorus ratios on the dominance of blue-green algae in Lake St. George, Ontario. Can. J. Fish. Aquat. Sci. 44: 598–604.

NAUWERCK, A. 1963. Die Beziehungen Zwischen Zooplankton und Phytoplankton in see Erken. Symb. Bot. Ups. 17: 163 p.

PAERL, H.W., AND J.F. USTACH. 1982. Blue-green algal scum: an explanation for their occurrence during freshwater blooms. Limnol. Oceanogr. 27: 121–217.

REUTER, J.G., AND R.R. PETERSEN. 1987. Micronutrient effects on cyanobacterial growth and physiology. N.Z. J. Mar. Freshwater Res. 21: 435–445.

ROBARTS, R.D., AND T. ZOHARY. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. N.Z. J. Mar. Freshwater Res. 21: 391–399.

RÖNICKE, H. 1986. Beitrag zur fixation des molekularen stickstoffs durch planktische Cyanophyceen in oinem dimiktischen, schwach durchflossenen Standgewässer. Ph.D. thesis, Humboldt-Universität, Berlin. 129 p.

SCHINDLER, D.W. 1974. Eutrophication and recovery in experimental lakes: implications for lake management. Science (Wash., D.C.) 184: 879–899.

SCHINDLER, D.W. 1975. Whole-lake eutrophication experiments with phosphorus, nitrogen, and carbon. Verh. Int. Ver. Theor. Angew. Limnol. 19: 3221–3231.

SCHINDLER, D.W. 1977. Evolution of phosphorus limitation in lakes: natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. Science (Wash., D.C.) 195: 260–262.

SCHINDLER, D.W., F.A.J. ARMSTRONG, S.K. HOLMGREN, AND G.J. BRUNSKILL. 1971. Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by additions of phosphate and nitrate. J. Fish. Res. Board Can. 28: 1763–1782.

SCHINDLER, D.W., H. KLING, R.V. SCHMIDT, J. PROKOPOWICH, V.E. FROST, R.A. REID, AND M. CAPEL. 1973. Eutrophication of Lake 227 by additions of phosphate and nitrate. Part 2. The second, third and fourth years of enrichment, 1970, 1971, 1972. J. Fish. Res. Board Can. 30: 1415–1440.

SCHINDLER, D.W., R.H. HESSLEIN, AND M.A. TURNER. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 26–33.

SHAPIRO, J. 1990. Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH. Verh. Int. Ver. Theor. Angew. Limnol. 24: 38–54.

SHEARER, J.A. 1978. Two devices for obtaining water samples integrated over depth. Can. Fish. Mar. Serv. Tech. Rep. 772: 9 p.

SMITH, V.H. 1983. Low nitrogen to phosphorus ratios favour dominance by blue-green algae in lake phytoplankton. Science (Wash., D.C.) 221: 669–671.

SMITH, V.H. 1990. Nitrogen, phosphorus, and nitrogen fixation in lacustrine and estuarine ecosystems. Limnol. Oceanogr. 35: 1852–1859.

STAINTON, M.P., M.J. CAPEL, AND F.A.J. ARMSTRONG. 1977. The chemical analysis of freshwater. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

STEWART, W.D.P., G.P. FITZGERALD, AND R.H. BURRIS. 1967. *In situ* studies on N_2-fixation using the acetylene reduction technique. Proc. Nat. Acad. Sci. (Wash., D.C.) 58: 2071–2078.

STEWART, W.D.P., G.P. FITZGERALD, AND R.H. BURRIS. 1968. Acetylene reduction by nitrogen-fixing blue-green algae. Arch. Mikrobiol. 62: 336–348.

TILMAN, D., R. KIESLING, R. STERNER, S. KILHAM, AND F.A. JOHNSON. 1986. Green, blue-green and diatom algae: taxonomic differences in competitive ability for phosphorus, silica, and nitrogen. Arch. Hydrobiol. 106: 473–485.

TOETZ, D., AND M. MCFARLAND. 1987. Lake loading ratios (N:P) and lacustrine nitrogen fixation. Water Resour. Bull. 23: 239–241.

VOLLENWEIDER, R.A. 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Tech. Rep. O.E.C.D. Paris Das/CSI/68.27: 182 p.

WOLFE, B., H.J. KLING, G.J. BRUNSKILL, AND P. WILKINSON. 1994. Multiple dating of a freeze core from Lake 227, an experimentally fertilized lake with varved sediments. Can. J. Fish. Aquat. Sci. 51: 2274–2285.

ZEVENBOOM, W., AND L.R. MUR. 1980. N_2-fixing cyanobacteria: why they do not become dominant in Dutch hypertrophic lakes, p. 123–130. *In* J. BARICA AND L.R. MUR [ed.] Hypertrophic ecosystems. Dr. W. Junk, The Hague, The Netherlands.

Aphanizomenon schindleri sp.nov.: a new nostocacean cyanoprokaryote from the Experimental Lakes Area, northwestern Ontario[1,2]

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Aphanizomenon schindleri sp.nov., a new nostocacean, bluegreen, cyanoprokaryote (cyanobacterium), is described from artificially eutrophied Canadian Shield lakes in the Experimental Lakes Area, northwestern Ontario. It is unique to low conductivity Canadian Shield waters to which nutrients have been added, and it became the dominant bloom species after years of phosphorus (P) and nitrogen (N) loading followed by N limitation (N:P ≤ 5:1 by mass) (Findlay et al. 1994. Can. J. Fish. Aquat. Sci. 51: 2254–2266). The new *Aphanizomenon* species appears to be most closely related to *A. gracile* but also resembles species of *Anabaena* with straight filaments. It differs from other species of *Aphanizomenon* in cell dimensions, length and width of akinetes and heterocytes, and in akinete and end cell shape.

Aphanizomenon schindleri sp.nov., un nouveau cyanoprocaryote nostocacé (une cyanobactérie) est décrit; il provient de lacs soumis à une eutrophisation artificielle qui sont situés dans la région des lacs expérimentaux, sur le Bouclier canadien, dans la partie nord-ouest de l'Ontario. Cette espèce n'est trouvée que dans les eaux à faible conductivité de lacs du Bouclier canadien auxquels des éléments nutritifs ont été ajoutés; elle est devenue l'espèce dominante dans les proliférations algaires après des années d'ajouts de phosphore (P) et d'azote (N) suivis d'une limitarion du N (rapport N:P ≤ 5:1 en masse) (Findlay et al. 1994. Can. J. Fish. Aquat. Sci. 51: 2254–2266). La nouvelle espèce d'*Aphanizomenon* paraît être le plus étroitement apparentée à *A. gracile*, mais elle ressemble aussi à des espèces d'*Anabaena* pourvues de filaments droits. Elle diffère des autres espèces d'*Aphanizomenon* par les dimensions de ses cellules, la longueur et la largeur des akinètes et des hétérocytes ainsi que par la forme des akinètes et des cellules terminales.

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An undefined species of cyanoprokaryote[3] (Nostocaceae), herein identified as *Aphanizomenon schindleri* sp.nov., initially appeared in 1976 in experimentally eutrophied Lake 227 (L227) of the Experimental Lakes Area (ELA), northwestern Ontario. It appeared 1 yr after the nitrogen (N) : phosphorus (P) ratio of added fertilizer was reduced from 13:1 to 5:1 (by mass) (Schindler 1977). It subsequently appeared in Lake 226 north (L226N) in 1978, following 5 yr of fertilization with carbon (C), N, and P at the ratio 10:5:1 (by mass). Both lakes supported an increased midsummer epilimnetic biomass of cyanoprokaryotes. Immediately following the onset of fertilization with the above 5:1 N:P ratio, *Anabaena planktonica* Brunnthal dominated in L226N and *Aphanizomenon gracile* Lemmermann dominated in L227. *Aphanizomenon schindleri* (first thought to be an *Anabaena*) was codominant with the two other species. By 1977, *A. schindleri* dominated the midsummer plankton in L227, and it is presently responsible for the majority of N_2-fixation in this lake (Findlay et al. 1994). It also became dominant in L226N, but at a much lower maximum biomass. Originally, this taxon was referred to as a form of *Anabaena levanderi* Lemmermann, but after detailed examination, comparison with similar taxa from the literature, and comparison with material from Scandinavia we concluded that it was an undescribed species of *Aphanizomenon*. The primary purposes of this paper are to describe this new nostocacean cyanoprokaryote, to document ecological data pertinent to it, and to describe its current distribution.

Methods

Brunskill and Schindler (1971) described the ELA, and Johnson and Vallentyne (1971) explained the rationale behind its existence. Phytoplankton samples have been analysed from the ELA since January 1969.

[1]This paper is one of a series done in honour of the 25th anniversary of the Experimental Lakes Area.

[2]For nomenclatural purposes, the actual month of publication of this issue of Can. J. Fish. Aquat. Sci. was February 1995.

[3]"Cyanoprokaryota" is now the preferred term for "bluegreen algae" and "cyanobacteria" because they are neither algae nor bacteria. The term is a compromise whose intent is to prevent further quarrelling between "algologists" and "microbiologists."

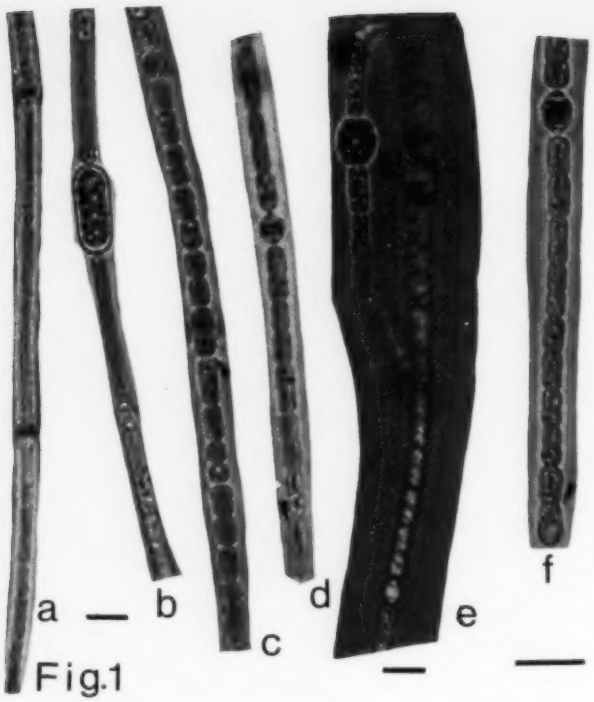

Fig.1

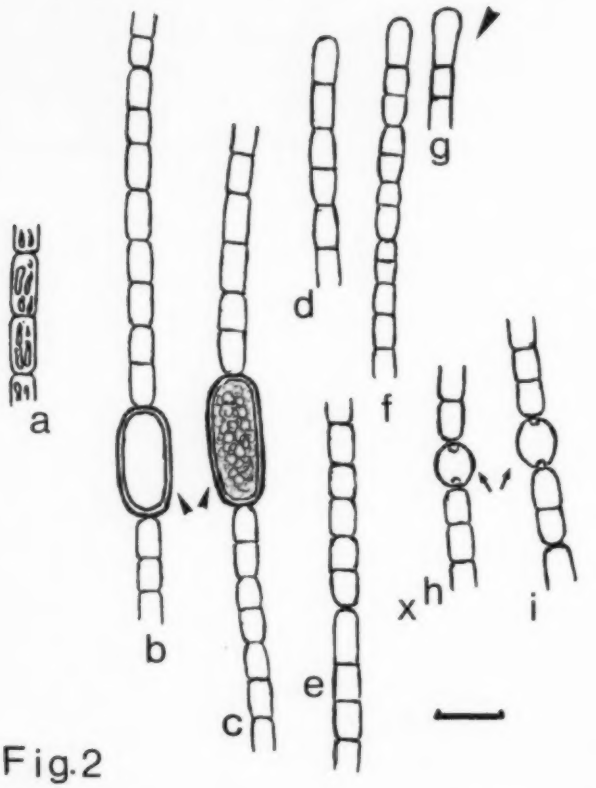

Fig.2

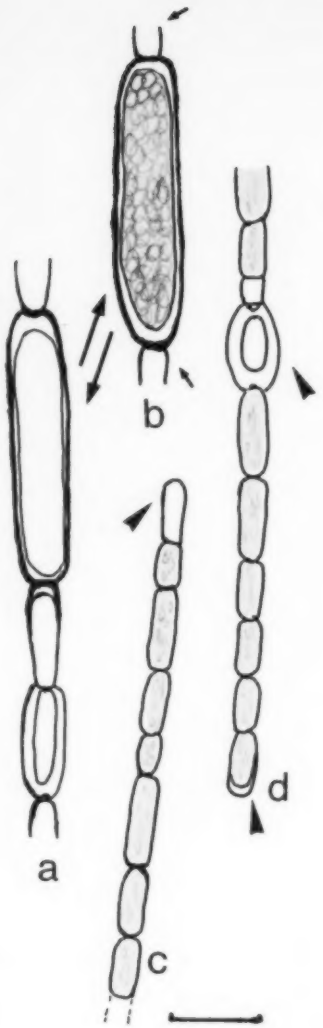

Fig.3

FIG. 1. Light photomicrographs of *Aphanizomenon schindleri* sp.nov. (a) A trichome with 2 heterocytes (arrows). (b) A mature akinete (arrow). (c) A trichome showing the position and character of the cells where fragmentation occurs (arrow). (d) A portion of

TABLE 1. Mean values for the taxonomic features measured in populations of *Aphanizomenon schindleri* sp.nov. in lakes 226 north (N) and 227, including results of *t*-tests indicating significant ($\alpha = 0.05$) differences in features between the two populations. Abbreviations for measured features: ENDL = end cell length, ENDTL = length of cell next to end cell, ENDTW = width of cell next to end cell, ENDW = width of end cell, FILLEN = length of filament, HTCAKN = no. of cells between heterocyte (= heterocyst) and akinete, HTCHTC = number of cells between heterocytes when there were more than one per filament, HTCL = heterocyte length, HTCW = heterocyte width, MATAKNL = mature akinete length, MATAKNW = mature akinete width, VEGL = vegetative cell length, VEGW = vegetative cell width. All measurements are in micrometers. SD = standard deviation; *Significant difference between lakes.

Feature	Number	Minimum	Maximum	Mean	SD
ENDL 227	41	4.2	12.6	7.2	2.0
ENDL 226N	36	4.2	12.6	7.25	1.7
ENDTL 227	37	4.2	11.3	7.3	1.9
ENDTL 226N	36	3.9	10.4	6.4*	1.6
ENDTW 227	37	1.4	4.2	2.6	0.56
ENDTW 226N	36	2.3	3.9	3.0*	0.49
ENDW 227	41	1.4	4.2	2.3	0.51
ENDW 226N	36	2.6	4.6	3.4*	0.49
FILLEN 227	59	98	951	338	204
FILLEN 226N	36	136	1280	503*	263
HTCAKN 227	40	0	26	8.6	5.7
HTCAKN 226N	36	0	38	8.9*	10.0
HTCHTC 227	40	14	41	24.4	6.6
HTCHTC 226N	36	24	72	41.8	14.2
HTCL 227	40	2.8	8.6	5.0	1.2
HTCL 226N	36	3.9	7.6	5.5*	0.9
HTCW 227	40	2.1	5.6	3.4	0.90
HTCW 226N	36	2.6	6.5	4.4*	0.91
MATAKNL 227	26	14	22.4	16.8	1.9
MATAKNL 226N	36	13	19.5	15.2*	2.3
MATAKNW 227	26	7	12.6	10.2	1.2
MATAKNW 226N	36	7.8	11.7	9.4*	0.92
VEGL 227	74	4.2	11.3	7.1	1.9
VEGL 226N	36	4.8	11.7	6.7	1.7
VEGW 227	74	1.6	4.2	2.6	0.58
VEGW 226N	36	2.3	3.9	3.1*	0.55

Material for this study came from phytoplankton samples taken during the routine sampling program described in Findlay (1978, 1981). Detailed measurements of features commonly used in species differentiation of filamentous cyanoprokaryotes (e.g., filament lengths; the lengths and widths of akinetes, heterocytes, vegetative cells, and end cells; see Table 1) were taken from populations in the two experimentally eutrophied lakes, L226N and L227, during periods when all features were present.

Morphometric analysis of the populations was used to discriminate between *Aphanizomenon* (Komárek 1958; Kovačik and Holeckova 1984; Komárek and Kovačik 1989; Watanabe 1991) and *Anabaena* (Komárkova 1988). All measurements, drawings, and photographs were made from material preserved in Lugol's solution. Significant differences ($P < 0.05$) between morphological features in the two populations and environmental parameters in the two lakes were determined by *t*-tests. Star diagrams (polygonal graphs; Dawidson 1947) were used to illustrate mean morphometric features of the populations simultaneously (see Komárek and Kovačik 1989 for *Aphanizomenon*). Taxonomic features listed in Table 1 were included; meristic features were not. The same data set used in the star diagrams was subjected to a cluster analysis (single linkage, distance equal to Pearson correlation) using SYSTAT (Wilkinson 1990).

Results and Discussion

Variability of the two most common species of *Aphanizomenon*, *A. flos-aquae* Ralfs ex. Bornet et Flahault and *A. gracile*, is well known (Komárek 1958; Horecka and Komárek 1979; Kovačik and Holeckova 1984; Komárek

a trichome showing the hyaline end cell (arrow). (e) A trichome with a gelatinous sheath (bottom arrow) and an immature akinete (top arrow). (f) A trichome showing a shorter, less hyaline end cell than in Fig. 1d (arrow). Scales: Fig. 1a = 10 μm, Figs. 1b–1f = 5 μm. FIG. 2. Camera lucida drawings of *Aphanizomenon schindleri* sp. nov. (a) Vegetative cells. (b) Vegetative cells and akinete (arrow). (c) Vegetative cells and akinete (arrow). (d, f, and g) Variation of vegetative growth and end cells with a swollen end cell (arrow). (e) Filament fragmentation. (h and i) Heterocytes (arrows) and vegetative cells. Scale = 10 μm. FIG. 3. Camera lucida drawings of *Aphanizomenon gracile* (Lake 227 population). (a,b) Akinetes (large arrows). (b) Akinete with cup-shaped extensions (small arrows). (c) Vegetative filament with empty end cell (arrow). (d) Vegetative filament with heterocyte (top arrow) and filled end cell (bottom arrow). Scale = 10 μm.

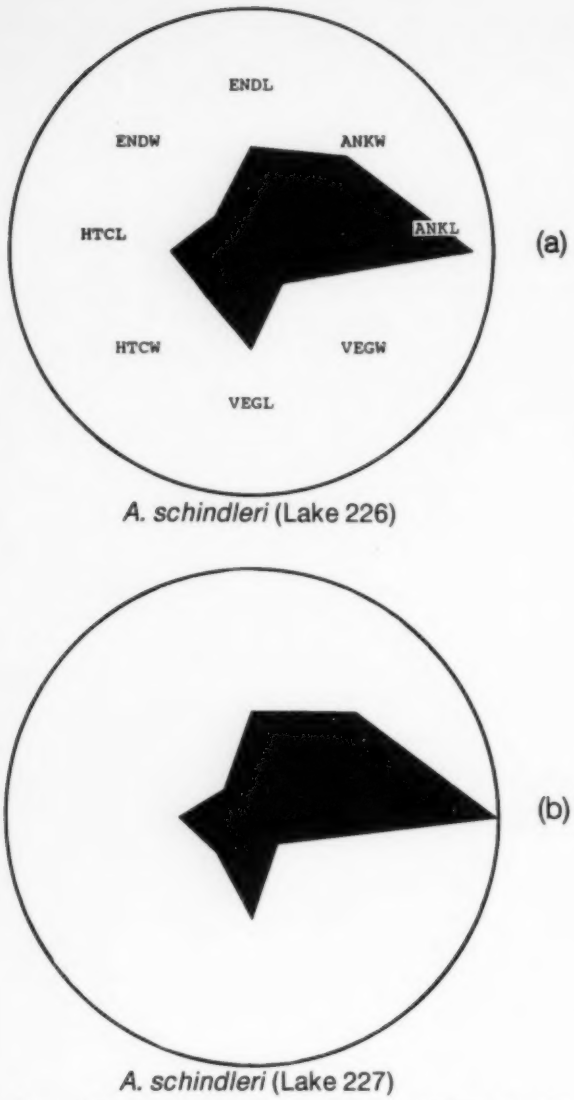

FIG. 4. Star diagram for mean morphometric features of *Aphanizomenon schindleri* sp.nov. from (a) Lake 226N and (b) Lake 227. Abbreviations for taxonomic features as in Tables 1 and 2.

and Kovačik 1989). Komárek and Anagnostidis (1989) proposed guidelines for classification of genera of the nostocacean cyanoprokaryotes based on morphological, physiological, and molecular characters. However, only morphological data are considered in this paper. The position of heterocyte development in relation to end cells and akinetes is one of the key features used to separate the genera *Anabaena* and *Aphanizomenon*. This feature is subsymmetric (occurs at irregular intervals) for *Aphanizomenon* and metameric (occurs at regular intervals) for *Anabaena* (Komárek and Anagnostidis 1989). *Aphanizomenon* spp. generally have unpigmented, elongate, terminal end cells, whereas *Anabaena* spp. usually have pigmented, undifferentiated end cells. However, there may be a noticeable degree of variability in the elongation and pigmentation, depending on the age of the filament (H.J. Kling, unpublished data).

General Morphological Features

Several taxonomically important features were measured and compared within and between populations of *A. schindleri* sp.nov. (Table 1). Photographs from the light microscope (LM) and a drawing illustrate the appearance of this species (Figs. 1, 2).

Solitary, more or less straight, free-floating filaments of varying length may or may not be encased in mucilage. The pale blue–green vegetative cells are short and barrel-shaped; they have a circular cross-section. Akinetes are oval to cylindrical–oblong in shape; heterocytes are round in young filaments to oval–cylindrical in older filaments. Both the akinetes and heterocytes are intercalary (never terminal) and are never situated beside one another. One or two akinetes in a row are positioned from one to several cells from a heterocyte. Usually there is one heterocyte per filament but in long filaments there may be two or three (rarely up to five). Filaments have a unique end cell (usually hyaline), which is elongate with a slightly swollen tip (Figs. 1d, 1f; 2d, 2f, 2g), similar to *A. gracile* (Komárek and Kovačik 1989). Fragmentation of the trichomes occurs at slightly swollen cells within the filament (Figs. 1c; 2d, 2f, 2g). This type of filament fragmentation is evident in several nostocacean taxa (e.g., *Anabaena* and *Aphanizomenon gracile*; Komárek and Kovačik 1989), and elongate slightly swollen end cells are characteristic of some species of *Aphanizomenon* (e.g., *A. gracile*). Vegetative cells within the trichomes are typically 2–2.5 times longer than wide. Empty terminal cells and the subsymmetric position of heterocyte development in relation to end cells and akinetes are characteristic of the genus *Aphanizomenon*.

The species is named in honor of Dr. David W. Schindler (University of Alberta, Edmonton, AB), who was the first leader of the ELA. Dr. Schindler designed and implemented the L226N and L227 N and P addition experiments that resulted in the discovery of the new species.

Aphanizomenon schindleri Kling, Findlay, and Komárek sp.nov.

Latin diagnosis: Filamenta libere natantia, recta vel minute flexuosa, exacte ad apices cylindrica, ad dissepimenta constricta. Cellulae apicales paucine elongatae, ± hyalinae, cylindricae vel haud capitatae.

Cellulae intercalares cylindricae, vacuolis gaseosis impletae, 4.2–11.7 μm * 1.6–4.2 μm. Heterocytae 1–3 (5 per trichoma), sphaericae vel ellipsoideae (2.8–8.6 μm * 2.1–6.5 μm). Akineta leviter curvata, cylindrica, elongata, apicibus rotundatis, 13–22.4 μm longae, 7–12.6 μm latae.

Holotypus: WPG 008, Freshwater Institute, Winnipeg.

Isotypus: National Museum, Ottawa, Canada depositus: Cana 38322.

Iconotypus: figura nostra 1.

Habitatio: In plancto lacubus meso - vel eutrophicus, Ontario meridionalis, Canada [locus classicus].

Etymologia: Species ad honoreus Prof. D.W. Schindler nominata.

The generic placement of these specimens was difficult because they have morphological characteristics resembling both *Anabaena* and *Aphanizomenon*. We initially identified

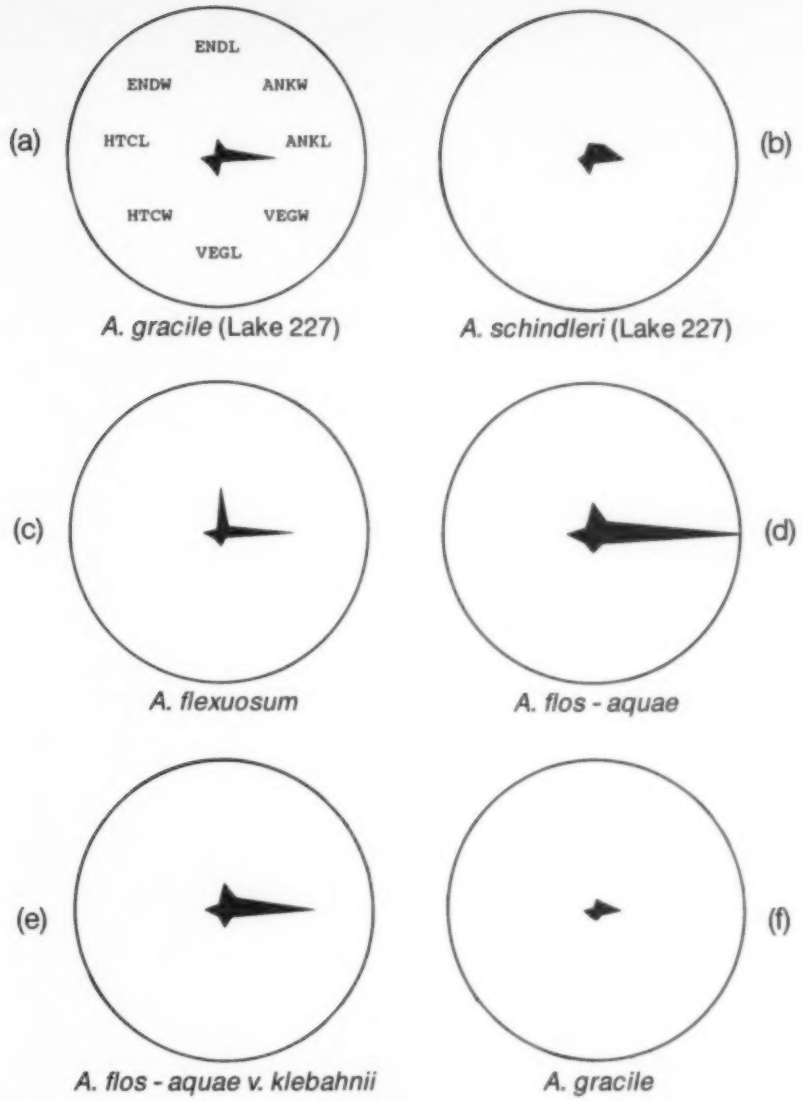

FIG. 5. Star diagram of (a) *Aphanizomenon gracile* and (b) *A. schindleri* sp.nov. from Lake 227 compared with (c) *A. flexuosum*, (d) *A. flos-aquae*, (e) *A. flos-aquae*, var. *klebahnii*, and (f) *A. gracile* from Czech localities. Abbreviations for taxonomic features as in Tables 1 and 2.

them as a form of *Anabaena levanderi* and thought that they belonged to the group of solitary straight *Anabaena*, i.e., *Anabaena elliptica* Lemmermann, *A. tenericaulis* Nygaard, and *A. danica* (Nygaard) Komárkova-Legnerova (Komárkova-Legnerova and Eloranta 1992). However, the swollen-tipped, elongate, hyaline end cells (Figs. 1d, 1f; 2d, 2e, 2g), the intercalary disintegration of trichomes at differentiated vegetative cells (Figs. 1c; 2e), and the positions of heterocytes and akinetes are features that place this taxon in the genus *Aphanizomenon* near *A. gracile*.

A low number of heterocytes (one or two per filament) is a typical feature of the type species *A. flos-aquae*, although *A. gracile* can develop several heterocytes, and most species of the genus *Anabaena* develop one to several heterocytes in a metameric position (Komárek and Anagnostidis 1989). In *A. schindleri*, the position of heterocyte development is subsymmetric in relation to akinetes and end cells, but like *A. gracile* it has one to five (usually one to three) heterocytes in a long filament. The frequency of heterocyte development is a nonconservative feature in both genera and it remains highly dependant on the degree of N limitation within the population (Levine and Lewis 1987; Findlay et al. 1994). However, the absence of heterocytes may also serve as a distinguishing feature between these genera because the addition of ammonia reduced heterocyte production to zero in *Aphanizomenon*, whereas *Anabaena* always had a few inactive heterocytes present (Schlangstedt et al. 1987). Maximum heterocyte frequency (five per filament) in the

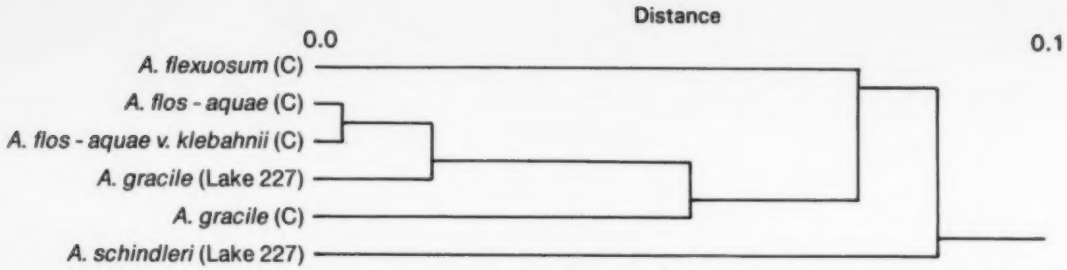

FIG. 6. Cluster diagram (using single linkage method) showing the relationship of the *Aphanizomenon* species in Fig. 5 to each other. Distance metric is 1 − Pearson correlation coefficient. C = Czech population.

TABLE 2. Comparisons of the dimensions of vegetative cells, terminal cells, heterocytes, and akinetes of *Aphanizomenon schindleri* and *A. gracile* from Lake 227 to four *Aphanizomenon* taxa from Czech populations studied by Komárek and Kovačik (1989). Abbreviations as in Table 1. ANKL = MATAKNL and ANKW = MATAKNW in Table 1.

Taxon	VEGL	VEGW	ENDL	ENDW	HTCL	HTCW	ANKL	ANKW
A. flos-aquae	8.9	6.25	14.65	4.65	12.15	5.7	68.55	8.55
A. gracile	4.6	2.85	5.35	1.9	5.85	4.15	11.25	4.9
A. flos-aquae var. *klebahnii*	8.2	4.85	12.15	3.65	8.9	4.05	41.4	7.85
A. flexuosum	6.4	3.05	21.25	2.7	8.2	3.95	33.55	4.65
A. schindleri	6.9	2.7	7.25	3.1	5.3	3.9	15.95	9.7
A. gracile	8.4	2.4	8.6	2.0	7.6	5.1	26.5	5.4

TABLE 3. Range of selected chemical parameters under which *Aphanizomenon schindleri* was present, and mean conditions at the time of maximum biomass. Cond = conductivity; DOC = dissolved organic carbon; TDN = total dissolved nitrogen; TDP = total dissolved phosphorus.

	Ca ($mg \cdot L^{-1}$)	Cl ($mg \cdot L^{-1}$)	Cond ($\mu S \cdot cm^{-1}$)	DOC ($\mu m \cdot L^{-1}$)	Fe ($mg \cdot L^{-1}$)	Mg ($mg \cdot L^{-1}$)	NH4 ($\mu g \cdot L^{-1}$)	NO3 ($\mu g \cdot L^{-1}$)	pH	TDN ($\mu g \cdot L^{-1}$)	TDP ($\mu g \cdot L^{-1}$)
Minimum	1.7	0.3	19	433	0.04	0.5	7	3	6.7	239	4
Maximum	2.3	0.9	44	2250	0.31	0.7	120	51	9.2	774	18
Mean	1.9	0.44	40	1290	0.08	0.61	20	6	8.9	583	11

TABLE 4. Selected mean chemical and biological parameters that were significantly (t-test, $\alpha = 0.05$) different in Lake 226 north (N) and Lake 227. Cond = conductivity; DOC = dissolved organic carbon; TDN = total dissolved nitrogen; TDP = total dissolved phosphorus.

Lake	Biomass ($\mu g \cdot L^{-1}$)	NH4 ($\mu g \cdot L^{-1}$)	Cond ($\mu S \cdot cm^{-1}$)	DOC ($\mu m \cdot L^{-1}$)	pH	TDN ($\mu g \cdot L^{-1}$)	TDP ($\mu g \cdot L^{-1}$)	Mg ($mg \cdot L^{-1}$)
226N	5533	17.4	23.2	515.8	7.0	310	5.6	0.52
227	41517	58.6	38.8	1567.5	8.2	768	11.8	0.64

populations of *A. schindleri* that we studied was greatest after N_2-fixation became undetectable near the end of the seasonal bloom (Findlay et al. 1994).

Aphanizomenon schindleri appears morphologically to be most similar to *A. gracile* but differs significantly in the dimensions of the vegetative and other prominent cells (i.e., length:width ratio of heterocytes and akinetes). It also differs in qualitative features such as the oval–oblong akinetes and bulbous tips on the end cells.

However, star diagrams (Figs. 4a and 4b) based on the means of conservative features measured (not including filament length or meristic characters) show similar patterns for the two populations. This tool, which uses a limited number of measurable morphometric features, has been shown by Komárek (1987) to be useful for species differentiation in chlorococcal greens as well as other *Aphanizomenon* taxa (Komárek and Kovačik 1989). Figure 5 compares the star patterns of *A. schindleri* and *A. gracile* from L227 to four *Aphanizomenon* taxa from Komárek and Kovačik (1989) using mean values for the characteristic features (Table 2). The star pattern of *A. schindleri* most closely matches the pattern of *A. gracile* (Fig. 5). A cluster diagram of the taxa in Table 2 shows their relationship to one another, based on the standardized measured features (Fig. 6). *Aphanizomenon gracile* (L227) sits between *A. flos-aquae*, *A. flos-aquae* var. *klebahnii* Elenken, and *A. gracile* (Czech population). *Aphanizomenon schindleri* and *A. flexuosum* Komárek and Kovácik appear quite separate. Although the dimensions of *A. gracile* (L227 population) were not identical to *A. gracile* (Czech population), the L227 taxon dominant in 1975 (Fig. 3) has been identified as *A. gracile* because its akinetes have cup-like extensions at both ends (Figs. 3a and 3b), a unique

feature of this species. A detailed morphometric study of North American and European *Aphanizomenon* populations may indicate that there should be a "gracile" complex of *Aphanizomenon*, which is characterized by cup-like extensions on the akinetes, but this matter is beyond the scope of this paper.

Aphanizomenon schindleri was found over a wide range of environmental conditions (Table 3). The length and width of cells, cell number, heterocyte number, filament length, and presence or absence of mucilage of filamentous cyanoprokaryotes vary in response to environmental conditions (Stulp 1982; Cmiech et al. 1984, 1986), which was the case in these populations (Table 4 cf. Table 1). Features such as end cell length, number of cells between heterocytes, and vegetative cell length (Table 1) did not vary between the two populations so these features have the greatest taxonomic value.

Periodicity and Ecology

Aphanizomenon schindleri is perennial and can co-occur with *A. gracile* and *Anabaena planktonica*. In both L227 and L226N, *A. schindleri* began as a codominant and eventually became the dominant N_2-fixer. It has been found at temperatures between 15–23°C, and reaches maximum development in June and August at water temperatures >18°C and pH >8.2. Maximum heterocyte production occurred early in the population following maximum measured N_2-fixation (1.751 $\mu g\ N \cdot L^{-1} \cdot h^{-1}$ (Findlay et al. 1994), which can occur at very low light levels (Hendzel et al. 1994). Maximum production of akinetes occurred in late August–September (H.J. Kling and D.L. Findlay, unpublished data).

Aphanizomenon schindleri has also been identified in mixed cyanoprokaryote populations of medium-sized Canadian Shield lakes in the Red Lake area, northwestern Ontario (Musclow Lake, Linge Lake). These lakes have neutral pH and slightly higher conductivity (70 $\mu S \cdot cm^{-1}$) than the ELA lakes and, in general, are less nutrient limited than the smaller ELA lakes. However, by midsummer they also become somewhat N_2 limited (S. Guildford, Department of Fisheries and Oceans, Winnipeg, Man., personal communication), and they develop mixed populations of N_2-fixing nostocacean cyanoprokaryotes, including *A. schindleri* (H.J. Kling, unpublished data).

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References

Brunskill, G.J., and D.W. Schindler. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139–155.

Cmiech, H.A., C.S. Reynolds, and G.F. Leedale. 1984. Morphological and ultrastructural variability of planktonic Cyanophyceae in relation to seasonal periodicity. I. *Gloeotrichia echinulata*: vegetative cells, polarity, heterocysts, akinetes. Br. Phycol. J. 19: 259–275.

Cmiech, H.A., C.S. Reynolds, and G.F. Leedale. 1986. Morphological and ultrastructural variability of planktonic Cyanophyceae in relation to seasonal periodicity. II. *Anabaena solitaria*: vegetative cells, heterocysts, akinetes. Br. Phycol. J. 21: 81–92.

Dawidson, J.F. 1947. The polygonal graph for simultaneous portrayal of several variables in population analysis. Madrono 9: 195–210.

Findlay, D.L. 1978. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1974 to 1976. Can. Fish. Mar. Serv. MS Rep. 1466: 41 p.

Findlay, D.L. 1981. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1977 to 1979. Can. MS Rep. Fish. Aquat. Sci. 1627: 40 p.

Findlay, D.L., R.E. Hecky, L.L. Hendzel, M.P. Stainton, and G.W. Regehr. 1994. Relationship between N_2-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. Can. J. Fish. Aquat. Sci. 51: 2254–2266.

Hendzel, L.L., R.E. Hecky, and D.L. Findlay. 1994. Recent changes of N_2-fixation in Lake 227 in response to reduction of N:P loading ratio. Can. J. Fish. Aquat. Sci. 51: 2247–2253.

Horecka, M., and J. Komárek. 1979. Taxonomic position of three planktonic blue-green algae from the genera *Aphanizomenon* and *Cylinderospermopsis*. Preslia/Praha, 51: 289–312.

Johnson, W.E., and J.R. Vallentyne. 1971. Rationale, background, and development of experimental lake studies in northwestern Ontario. J. Fish. Res. Board Can. 28: 123–128.

Komárek, J. 1958. Die Taxonomische Revision der planktischen Blaualgen der Tschechoslowakei, p. 10–206. *In* J. Komárek and H. Ettl [ed.] Algologische Studien. Nakl. CSAV, Praha.

Komárek, J. 1987. Species concept in coccal green algae. Arch. Hydrobiol. Suppl. 45: 437–471.

Komárek, J., and K. Anagnostidis. 1989. Modern approach to the classification system of cyanophytes. 4-Nostocales, Stigonematales. Arch. Hydrobiol. Suppl. 56: 247–345.

Komárek, J., and L. Kovačik. 1989. Trichome structure of four *Aphanizomenon* taxa (*Cyanophyceae*) from Czechoslovakia, with notes on the taxonomy and delimitation of the genus. Plant Syst. Evol. 164: 47–64.

Komárkova, J. 1988. Morphological variation in natural populations of *Anabaena lemmermannii* in respect to existence of *Anabaena utermoehlii*. Arch. Hydrobiol. Suppl. 50–53: 93–108.

Komárkova-Legnerova, J., and P. Eloranta. 1992. Planktic blue-green algae (Cyanophyta) from Central Finland (Jyvaskyla region) with special reference to the genus *Anabaena*. Arch. Hydrobiol Suppl. 67: 103–133.

Kovačik, L., and H. Holeckova. 1984. Biometric evaluation of populations of *Aphanizomenon flos-aquae* from Trebon pond basin (Czechoslovakia). Arch. Hydrobiol. Suppl. 50–53: 303–314.

Levine, S.N., and W.M. Lewis. 1987. A numerical model of nitrogen fixation and its application to Lake Valencia, Venezuela. Freshwater Biol. 17: 265–274.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. Science (Wash., D.C.) 195: 260–262.

Schlangstedt, M., P.S. Bisen, G. Dudel, and J.G. Kohl. 1987. Interaction of combined nitrogen availability and light in the regulation of growth, heterocyst differentiation and nitrogen fixation of planktic bluegreen algae *Anabaena solitaria* Kleb. Arch. Protistenkd. 134: 389–396.

Stulp, B.K. 1982. Morphological variability of *Anabaena* strains (Cyanophyceae) under different culture conditions. Arch. Hydrobiol Suppl. 31: 165–176.

Watanabe, M. 1991. Studies on the planctonic blue-green algae 3. Some *Aphanizomenon* species in Horraido, northern Japan. Bull. Nat. Sci. Mus. (Tokyo), Ser. B 17(4): 141–150.

Wilkinson, L. 1990. SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, Ill.

Multiple Dating of a Freeze Core from Lake 227, an Experimentally Fertilized Lake with Varved Sediments[1]

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A freeze core taken from Experimental Lakes Area Lake 227 in 1988 contained 321 rhythmically paired, dark and light laminations in the upper 60.7 cm. Tape peels revealed cyclic, seasonal abundance peaks in organic and inorganic remains, which suggested that the couplets are varves. However, comparison between varve chronology and 22 yr of experimental changes in phosphorus (P) and nitrogen (N) loading and their influence on the planktonic community confirmed that the most recent varve-year estimates were 5 or 6 yr too old; this was caused by irregular sedimentation and multiple algal blooms resulting from experimental fertilization since 1969, and indistinct laminations that hampered precise couplet identification and separation. Dated horizons determined from biostratigraphic markers were used to generate compatible profiles between 1-cm slices of Lake 227 ^{137}Cs flux and reference fallout records. Nutrient concentration profiles were less helpful, as increases in carbon, N, and, P were gradual and no distinct horizon was identified as a clear marker of eutrophication. Long-term assessment of the varve chronology using ^{210}Pb was hindered by experimental additions of ^{226}Ra to the lake in 1970, although similar sedimentation rates from varve years 1860–1934 suggested that the varve and the deep part of the ^{210}Pb chronologies were comparable.

Une carotte obtenue par cryoprélèvement en 1988 au fond du lac 227, du secteur des lacs expérimentaux, contenait 321 lamelles alternativement sombres et claires dans les 60,7 cm supérieurs. Des échantillons détachés au moyen d'un ruban gommé ont révélé l'existence de pics d'abondance saisonniers et cycliques qui se manifestent dans les restes organiques et inorganiques; cela donne à penser que les paires de lamelles forment des varves. Toutefois, la comparaison entre la chronologie varvaire et les relevés de 22 ans sur les changements expérimentaux de la charge en phosphore (P) et en azote (N) ainsi que de leur influence sur la communauté planctonique, a permis de confirmer que les estimations des années varvaires les plus récentes étaient 5 ou 6 ans trop vieilles; cela était attribuable à une sédimentation irrégulière, à la formation de multiples proliférations algaires causées par la fertilisation expérimentale, ainsi qu'à la présence de lamelles indistinctes qui ont nui à l'identification et à la séparation précises des paires de lamelles. Les horizons, dont l'âge a été déterminé à partir de marqueurs biostratigraphiques, ont permis de constituer des profils par tranches de 1 cm d'épaisseur du flux de ^{137}Cs du lac 227 et compatibles avec les profils de référence des retombées. Les profils de concentration en éléments nutritifs ont été moins utiles puisque l'augmentation en C, en N ainsi qu'en P a été graduelle et qu'il a été impossible de déterminer des horizons distincts qui puissent servir de marqueurs clairs de l'eutrophisation. L'évaluation de la chronologie varvaire à long terme au moyen du ^{210}Pb a été compliquée par le fait qu'il y a eu des additions expérimentales de ^{226}Ra dans le lac en 1970 même si des taux similaires de sédimentation pour les années varvaires 1960–1934 indiquent que les varves et que les chronologies au ^{210}Pb des parties profondes sont comparables.

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Interpretation of lake sediment records from recent centuries contributes much to our understanding of the effects on lakes of natural or anthropogenic environmental change. However, an accurate time scale is needed before lacustrine deposits can be used to reconstruct histories of pollution, vegetation, soil erosion, or lake productivity. Many techniques are used to generate a chronology, including direct identification and enumeration of annual laminations and radiochemical methods (e.g., ^{137}Cs, ^{210}Pb).

Varves are "a sedimentary bed or lamina or sequence of laminae deposited in a body of still water within one year's time" (Bates and Jackson 1987, p. 719). Lakes that are deep relative to their surface areas are most likely to have varved

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sediments, particularly in the absence of sediment mixing caused by currents or zoobenthos (O'Sullivan 1983; Saarnisto 1986). In recent years, analyses of mineralogical, chemical, and biological contents of varved lake sediments have produced detailed paleolimnologic, paleoclimatic, and paleoenvironmental reconstructions (e.g., Perkins and Sims 1983; Dean et al. 1984; Renberg et al. 1984; see reviews by O'Sullivan 1983 and Saarnisto 1986).

In lake sediments that do not display annual deposits, ^{137}Cs and ^{210}Pb are commonly used to establish chronologies over the past 40 and 150 yr, respectively (see reviews by Ritchie and McHenry 1990; Robbins 1978; Oldfield and Appleby 1984a). ^{137}Cs in the environment originates from nuclear testing or nuclear reactors (Ritchie and McHenry 1990), and initial input of ^{137}Cs began in 1952 ±2 yr; measurable quantities in soils began in 1954. Significant global peaks of ^{137}Cs atmospheric fallout occurred in 1958 and 1963–64 during atmospheric nuclear weapons testing. Although ^{137}Cs profiles are often complicated by delayed input from the drainage basin soils, physical reworking of the sediment (e.g., Robbins and Edgington 1975; Robbins et al. 1978), and diffusion (e.g., Torgenson and Longmore 1984), onset and peaks in ^{137}Cs activity have been widely used as stratigraphic markers.

^{210}Pb, a natural radioactive element of the ^{238}U decay series, has a half-life of 22.26 yr. Various models have been developed to interpret the distribution in lake sediments of unsupported ^{210}Pb (i.e., ^{210}Pb that is separated from its parent isotopes and is used to determine the length of time passed since deposition). Linear profiles that relate an exponential decrease in unsupported ^{210}Pb with depth are interpreted using the constant flux – constant sedimentation rate model, where the slope of the line is used to calculate the sedimentation rate (Oldfield and Appleby 1984a). Nonlinearities are normally interpreted by assuming a constant net rate of supply of ^{210}Pb from the lake waters to the sediment, although changes in the sedimentation rate may have occurred. In this constant rate of supply model, troughs in the unsupported ^{210}Pb profile are interpreted as periods of elevated sedimentation because the ^{210}Pb concentration is diluted by excess mass flux (Oldfield and Appleby 1984a).

Sediments from Lake 227 of the Experimental Lakes Area (ELA) contain alternating light and dark laminations. We used multiple dating techniques to test the hypothesis that the laminated couplets in Lake 227 sediments are varves. Direct examination of laminations by light and scanning electron microscopy provided evidence for an annual cycle of sedimentation. The chronology established by counting couplets was then compared with independent time scales determined from biostratigraphic markers (i.e., siliceous algae, cyanophyte remains), geochemical profiles resulting from 22 yr of experimental fertilization, and radiochemical methods (^{137}Cs, ^{210}Pb).

Materials and Methods

Site History

Regional geology, limnology, and chemistry of the lakes of the ELA were described in early papers (Brunskill and Schindler 1971; Schindler 1971; Armstrong and Schindler 1971; Brunskill et al. 1971). Specifically, Lake 227 has a surface area of 5 ha and a maximum depth of 10 m; it is a dilute headwater lake that is fed mostly by precipitation and overland flow. Water loss occurs through one outlet and evaporation. The lake is generally meromictic; spring turnover is presently incomplete below 7 m and fall turnover is rarely complete below 9 m (Schindler et al. 1987). Anoxic conditions occur in the hypolimnion for most of the year (Schindler 1971). Many of these variables favor the deposition and preservation of annually laminated sediments of biogenic origin (O'Sullivan 1983).

The lake was experimentally fertilized with phosphoric acid and sodium nitrate beginning in 1969 (Schindler et al. 1971). Nitrogen (N) addition was terminated in 1990; phosphorus (P) is still being added. Initial loadings were 0.48 g $P \cdot m^{-2} \cdot y^{-1}$ and 6.29 g $N \cdot m^{-2} \cdot y^{-1}$ (Schindler et al. 1973). The ratio by mass of N to P from 1969 through 1974 was 13:1 and was reduced to 5:1 between 1975 and 1989.

Sediment Core Analyses

Sediments were sampled using a "letterbox" freeze-corer (Huttunen and Meriläinen 1978) in the deepest part of the lake during the summer of 1988. Sediment analyses used both nondestructive and destructive techniques. Initial nondestructive techniques focused on (1) identifying annual cycles of sedimentation, and (2) establishing a chronology based on varve counts that could provide a basis for radiochemical (^{137}Cs, ^{210}Pb) and geochemical (carbon (C), N, P) sampling.

Nondestructive Analyses

The frozen core was trimmed into a rectangular slab using a band saw. The cross-sectional surface was planed, rinsed with distilled water to improve the visibility of the laminations, and photographed. Tracings of the laminations were constructed from the photographs, enlarged, counted, and measured to the nearest 0.1 mm.

Freeze drying the core for 4 d produced a thin film of dry, powdery sediment that enabled tape peels to be prepared (Simola 1977; Davidson 1988). Sediment was collected on double-sided clear plastic tape, adhered to small microscope slides, and photographed. The laminations were sketched and their contents were examined using a Cambridge 100-90 Scanning Electron Microscope (1000 – 20 000 ×).

To supplement the tape peel data, small quantities of individual laminations were dispersed in distilled water, and either examined using an inverted microscope at 100–650 × or dried on 10-mm coverslips, mounted in Hyrax medium, and examined with a compound microscope. Replicate coverslips were gold coated and viewed using scanning electron microscopy at magnifications ranging from 1000 – 20 000 ×.

Destructive Analyses

Individual varve years from 1939–1977 were separated and weighed. Composite samples of five varve years were obtained from the remainder of the core. The area of the rectangular depositional surface and dry sediment weight were then used to determine sedimentation rates. Three-point running means were used to smooth accumulation-rate estimates because of the uncertainty of completely separating sediment couplets. Replicate samples yielded a 10% standard deviation of the mean (SD) for accumulation rates.

^{137}Cs was measured in individual varve years from 1947–1976 by methods detailed in Wilkinson (1985). Decay-corrected ^{137}Cs flux, determined from ^{137}Cs activity and smoothed sediment accumulation data, was compared with the ^{137}Cs fallout data (bulk precipitation) recorded at Pinawa,

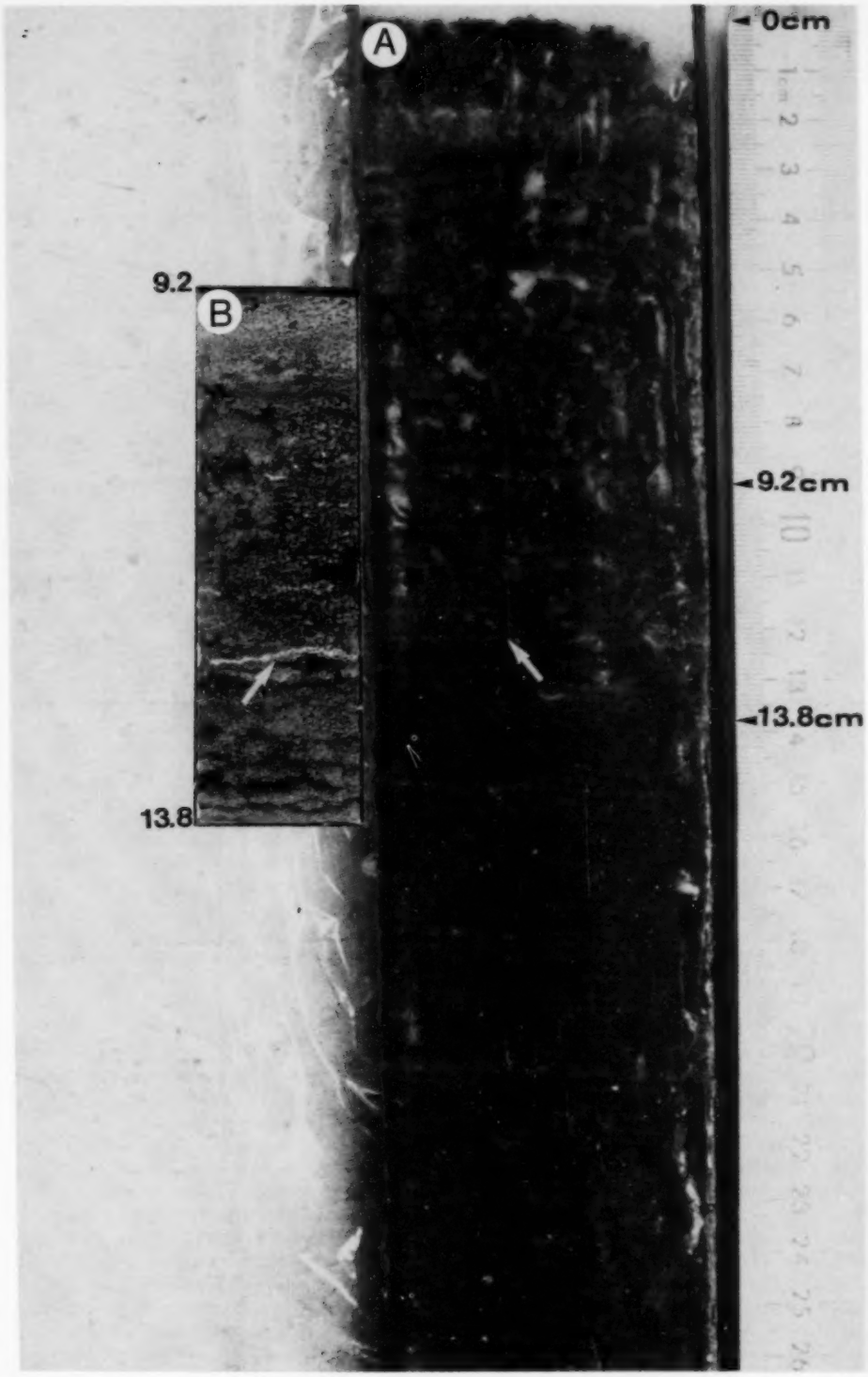

FIG. 1. (A) Upper section of Lake 227 freeze core. Couplets are composed of a thick, light-colored lamination overlain by a thin, dark-colored lamination. (B) Tape peel of cored interval 9.2–13.8 cm. The white arrows identify the 1971 diatom bloom visible in both A and B.

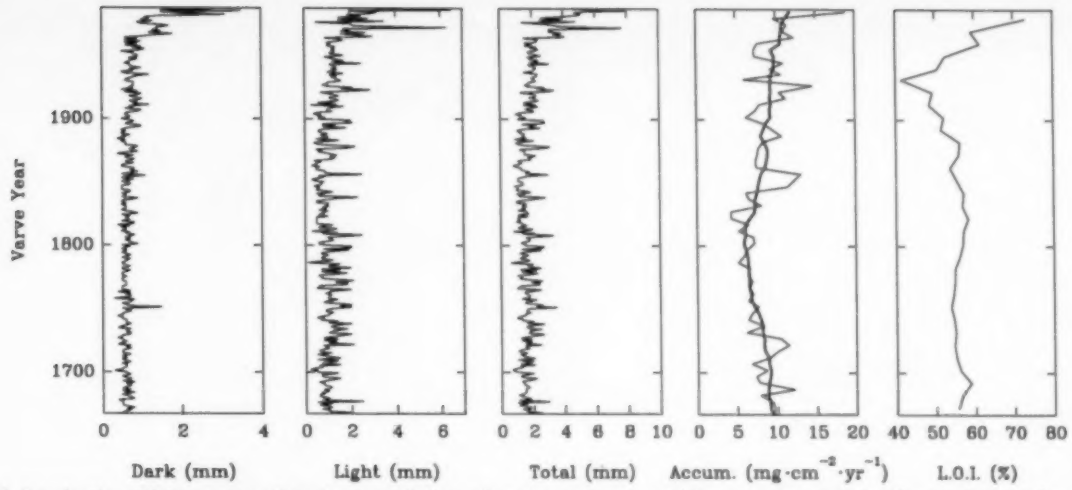

FIG. 2. Lamination thicknesses; sediment accumulation, plotted as averages of five varve years and a three-point running mean (bold); and loss-on-ignition (L.O.I.) profiles for a varve-year sequence of 321 years (1667–1987).

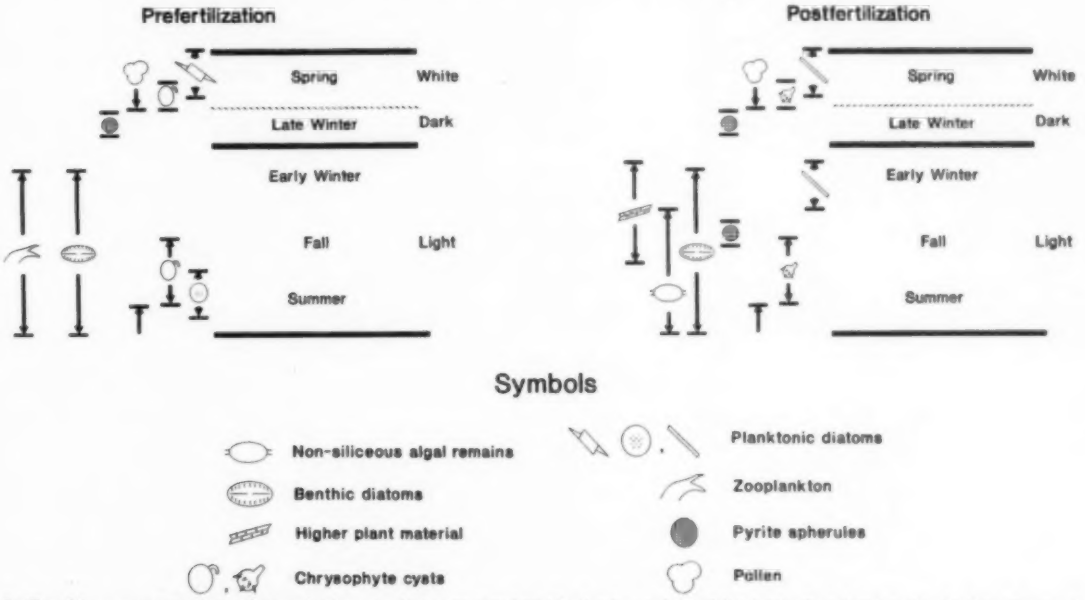

FIG. 3. Graphic representation of a typical varve in cross section displaying selected biological and mineralogical components and their seasonal distribution. Note that pollen distribution extends from spring through summer. Both pre- and post-fertilization models are shown. Not to scale.

Manitoba, which began in 1964. Cesium fallout data from New York City were used to extrapolate the Pinawa data to 1954 after multiplying by a factor of 0.98, the ratio of Pinawa to New York fallout from 1964 to 1976 (NRCC 1983).

Total ^{210}Pb activity was determined in 17 multiple varve-year samples spanning 1810–1982. Three samples from composite varve years 1800–1804, 1900–1904, and 1970–1971 were also analyzed for ^{226}Ra to estimate the supported ^{210}Pb content. Analytical methods followed Wilkinson (1985).

Carbon, N, and P were analyzed in individual varve years from 1939–1977, and in the composite samples of five varve years from the remainder of the core, by the methods of Stainton et al. (1977).

Results

General Core Description

Our short core (60.7 cm) yielded 321 couplets consisting of alternating light and dark horizontal laminations (Fig. 1). These sediments were undisturbed, contacts between laminations were generally sharp, and no gradation of grain size within couplets was identified, suggesting that episodic turbidity flows were rare.

Lamination thickness did not appreciably change throughout the core except near the surface (Fig. 2). Recent increases in lamination thickness were probably caused by lake fertilization and a lack of compaction. Below these strata, the

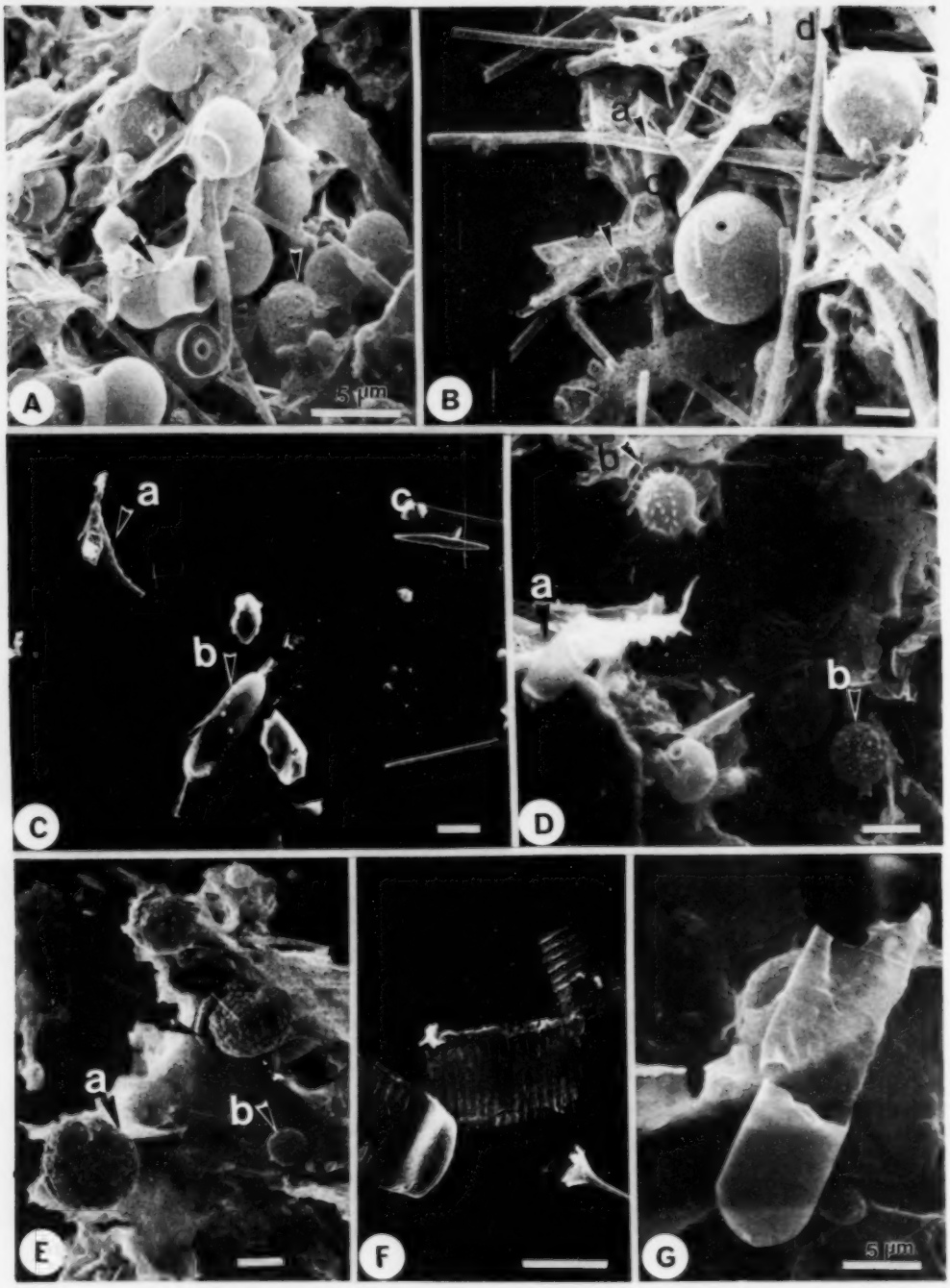

FIG. 4. White lamination from varve year 1973 for (A) *Uroglena volvox* (arrows) (scale = 5 µm) and (B) *Synedra* sp. (arrow a); *Mallomonas crassisquama* scale (arrow b); *Dinobryon cylindricum* cyst with a broken collar (arrow c); and an unidentified cyst (arrow d) (scale = 10 µm). (C) Light lamination from varve year 1973. Broken *Staurastrum* sp. (arrow a); *Apanizomenon gracile* akinete (arrow b); and a small bent valve of *Synedra* sp. (arrow c) (scale = 10 µm). (D) Light lamination from varve year 1971. *Apanizomenon* akinete (arrow a) and possibly *Uroglena* cysts (arrow b) (scale = 10 µm). (E) Dark lamination from varve year 1960. Pyrite spherules in framboidal aggregations often found at the upper dark–light contact (arrow a). *Cyclotella stelligera* (arrow b); these cells indicate summer–fall plankton (arrow b) (scale = 10 µm). Light laminations from varve year 1960 for (F) *Rhizosolenia longiseta* (scale = 10 µm) and (G) *Rhizosolenia* resting stage.

dark lamination thickness was relatively constant, ranging from 0.5 to 0.8 mm. In contrast, the light laminations were more variable, fluctuating from 0.5 to 2.0 mm in thickness. Light laminations appeared to dictate the overall couplet thickness.

Sediment accumulation rates ranged from 5 to 20 $mg \cdot cm^{-2} \cdot yr^{-1}$ throughout the last 321 varve years (Fig. 2). Data smoothed by a three-point running mean revealed that sedimentation decreased from the bottom of the core until the early 1800s and then increased to the present.

Organic matter, estimated from loss-on-ignition (Fig. 2), was relatively steady near 55% throughout most of the lower part of the core, but began to decrease between varve years 1850 and 1900, and attained a minimum near varve year 1930. Organic content was highest at the top of the core.

Annual Cycles of Sedimentation

Laminations in the tape peels from both pre- and post-fertilization years were examined qualitatively. Paired light and dark laminations appeared to represent annual deposits, based on cyclical peaks in organic and inorganic remains identified in the couplets. These remains included diatoms and chrysophyte cysts, which sometimes produced a distinct white lamination, and pyrite spherules, nonsiliceous algal remains, zooplankton body parts and eggs, and pollen (Fig. 3).

Light laminations from before fertilization were composed of summer planktonic taxa such as *Cyclotella bodanica*, *C. stelligera*, *Rhizosolenia eriensis*, *R. longiseta*, *Mallomonas duerrschmidtiae*, *M. crassisquama*, and desmids (Findlay and Kling 1975; Findlay 1978; Findlay 1981). Other siliceous and nonsiliceous algal remains included benthic diatoms, zooplankton remains, eggs, thecate amoebae, and some chrysophyte cysts and scales. Also present were brown detritus, most likely consisting of inorganic mineral matter and degraded plant material, which may have been washed in during seasonal storms.

In the uppermost couplets representative of postfertilization varve years, light laminations were composed primarily of chrysophyte cysts and scales (*Uroglena volvox*, Fig. 4A; *Synura* spp., *Mallomonas crassisqauma*, and *M. pseudocoronata*), diatoms (*Synedra* spp.; *Tabellaria flocculosa*; *C. stelligera*, Fig. 4E), desmids (*Staurastrum*, Fig. 4C), cyanobacterial filaments (*Oscillatoria redekei*, Fig. 5E), and later akinetes of N_2-fixers (*Aphanizomenon*, Fig. 4C, D; *Anabaena*). These taxa occur only in summer (Findlay and Kling 1975; Fig. 3) and confirm that the light laminations were deposited at this time.

Dark laminations, both from postfertilization strata and from deeper in the core, contained benthic algae along with sparse deposits of algal and zooplankton remains. Pyrite spherules (Fig. 5D), framboidal aggregations (Fig. 4E), and abundant organic matter near the light–dark contact, were responsible for the dark appearance of the lamination. Based on its stratigraphic position, the dark lamination portion of the couplet probably represented deposition during the late winter (Fig. 3). The presence of some summer biota in these laminations may be a result of continued growth under thin ice cover and subsequent settling from nonturbulent waters.

The tnin white laminations near the upper contact of the dark lamination were rarely visible to the unaided eye, but under the light microscope were more evident, particularly below 15 cm depth in prefertilization varve years. Often, there were two or three distinct laminations within the contact zone, consisting of a lamination of chrysophyte cysts overlain by a layer of diatoms and followed by another layer of cysts. Pollen grains did not form distinctive microlaminations, but instead were dispersed within the cyst and diatom layers. Pollen concentration decreased upwards through the light lamination. These data are indicative of spring deposition (Fig. 3).

Sediment Chronology

Core description and biological evidence for annual cycles of accumulation suggested that the sediments contained a continuous record of 321 varve years. However, the laminations were difficult to trace, particularly in the upper few centimetres and at some deeper intervals, which probably led to errors in age estimates. Independent biostratigraphic markers were required to evaluate the magnitude of these potential errors in the sediment deposited after lake fertilization, so historic plankton records were compared with the varve chronology. Couplet counts were also compared to ^{137}Cs, ^{210}Pb, C, N, and P profiles.

Biostratigraphic Markers

Nonsiliceous algae dominated the phytoplankton community following fertilization in 1969; some of these taxa could be used as biostratigraphic markers. Several major phytoplankton taxa showed distinctive postfertilization changes; for example, desmids dominated in 1969, *Synedra* spp. bloomed in June 1971, and *Aphanizomenon gracile* appeared in August 1975 (Kling and Holmgren 1972; Schindler et al. 1973; Findlay and Kling 1975; Findlay 1978, 1981, 1983). Individual varve years contained evidence of these historical events.

Between late summer 1969 and 1974, *Oscillatoria redekei* was prominent in the plankton (Schindler et al. 1973; Findlay and Kling 1975), yet it is first recorded in the tape peels at varve year 1963 (marker A). Second, distinctive layers of *Synedra* (Figs. 5A and 5C) occurred starting in varve year 1965, which most likely corresponded to June 1971 (Schindler et al. 1973; Findlay and Kling 1975) (marker B). In sediments below this, the same varve consisted of microlayers of chrysophytes (*U. volvox*, *Dinobryon bavaricum*), scales (*M. duerrschmidtiae*, *M. crassisquama*), filamentous cyanobacterial remains, and algal remains typically found only in the previous prefertilization varves. Third, the dark lamination of varve year 1971 contained *Aphanizomenon* akinetes, although these sediments must have been deposited in 1975, or later, because *Aphanizomenon* taxa were absent prior to 1975 (Findlay 1978) (marker C). *Aphanizomenon* taxa, present in the 1975 plankton, were dominated initially by *A. gracile* (Findlay 1981). Its akinetes are easily distinguishable from akinetes of other species, and were the predominant akinete found in the varve year 1971 sediment sample.

In summary, three marker beds demonstrated that the most recent varve chronology was ≈5 or 6 yr too old. Although the planktonic record indicated that varve year 1963 (marker A) could have been deposited between 1969 and 1974, it may represent 1969 because varve year 1965 (marker B) was 1971. In addition, varve year 1971 (marker C) represented at least 1975.

^{137}Cs Chronology

The decay-corrected ^{137}Cs record for varve years from 1947–1976 was quite irregular; several years were below analytical limits (e.g., 1954, 1961, 1970), and no distinct

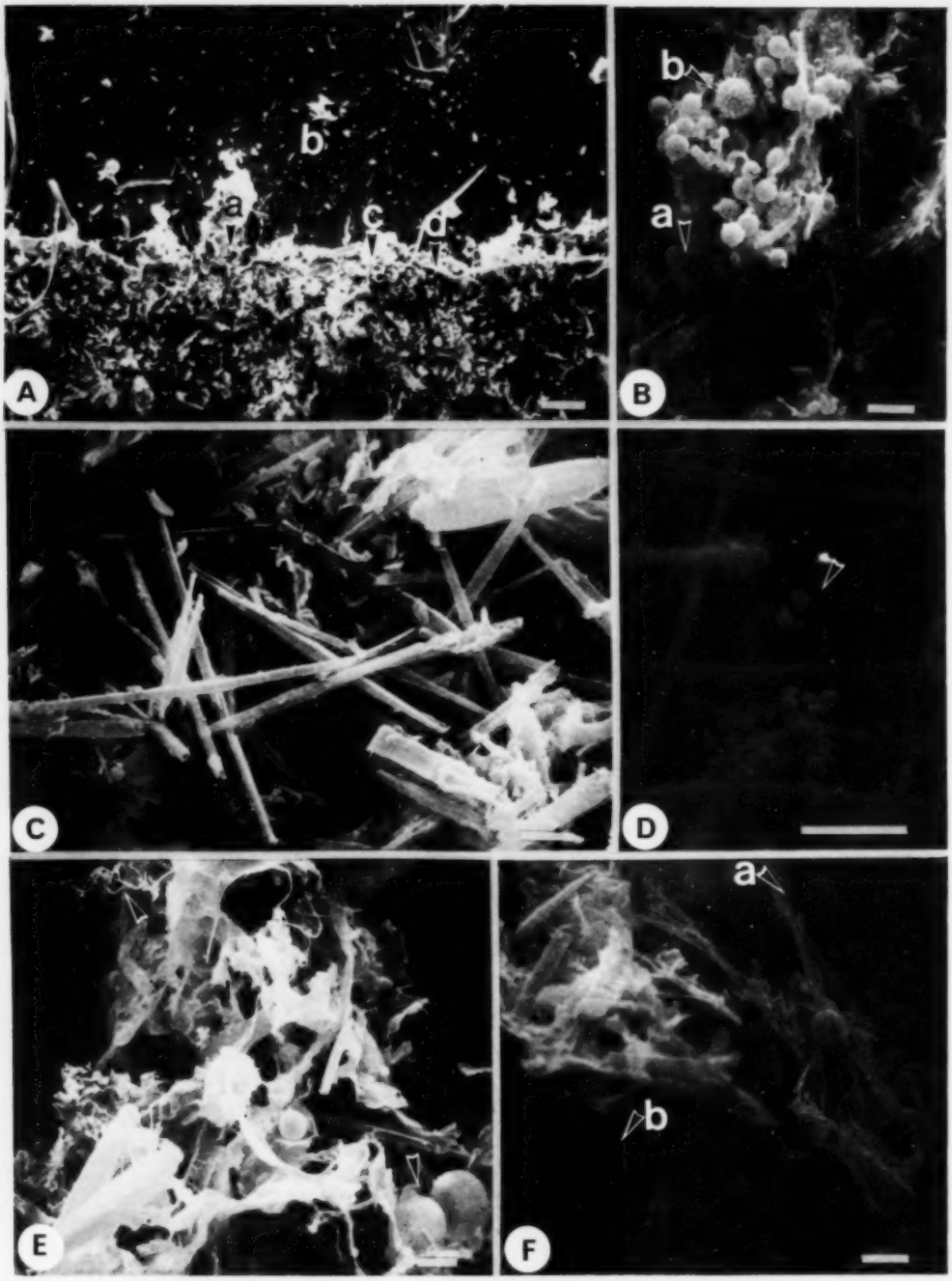

FIG. 5. (A) Thick (≈500 μm) white lamination (arrow b, indicating vertical strip between arrows a and c) from varve year 1965 containing *Synedra* sp. The phytoplankton record indicates that this stratum represents June 1971 (see text for an explanation of the discrepancy in years) (scale = 125 μm). (B) Cyst layer (Fig. 5A, area indicated by arrows c and d) immediately preceding the large *Synedra* lamination (Fig. 5A, arrow b). *Spiniferomonas trioralis* Takahashi cyst (arrow a) and *Mallomonas* cyst (arrow b) (scale = 50 μm). (C) Expanded view of lamination between arrows a and c in Fig. 5A, showing the mass of *Synedra* (scale = 10 μm). (D) Microlamination of *Asterionella formosa* and *Rhizosolenia* with dispersed pyrite spherules (arrow) (scale = 10 μm). (E) Light lamination from about varve year 1966. Cyanobacterial filaments (arrow a) and *Dinobryon cylindricum* Imhof cyst (arrow b). (F) White lamination from about varve year 1966–1967. Cyst of *Chrysosphaerella longispina* Lauterborn (arrow a) and bristle of *C. longispina* (arrow b) (scale = 10 μm).

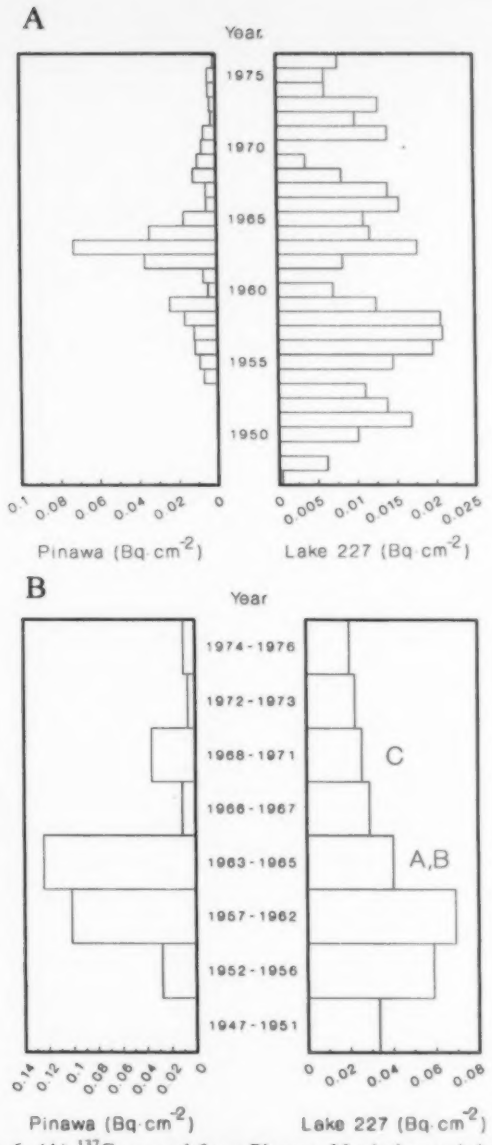

FIG. 6. (A) ^{137}Cs record from Pinawa, Manitoba, and decay-corrected ^{137}Cs flux profile of individual varve years from Lake 227. ^{137}Cs was not assayed below varve year 1947; analyses from another core indicate deeper penetration as a result of diffusion, although it is a very small fraction of the total ^{137}Cs in the sediment (J. Crusius and R.F. Anderson, University of British Columbia, Vancouver, B.C., personal communication). Note different horizontal scales. (B) Decay-corrected ^{137}Cs flux profile for Lake 227 replotted as 1-cm slices; determined by summing individual varve-year thicknesses. The corresponding Pinawa profile is also shown. Biostratigraphic markers A, B, and C from varve years 1963, 1965, and 1971 represent depositional years 1969, 1971, and at least 1975, respectively. Note different horizontal scales.

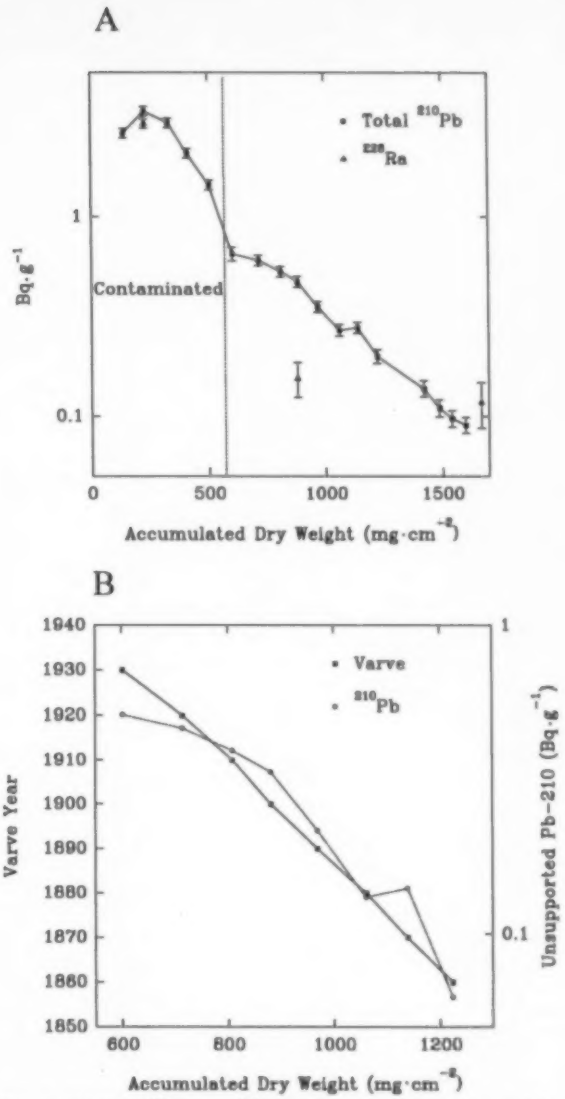

FIG. 7. (A) Total ^{210}Pb and ^{226}Ra versus accumulated dry weight. Samples left of dotted line are contaminated by experimental addition of ^{226}Ra in 1970. Error bars represent 2 SD. (B) Unsupported ^{210}Pb determined from uncontaminated samples, and varve year versus accumulated dry weight.

peak could be identified (Fig. 6A). There was no clear correspondence with the fallout record. To approximate the standard sample resolution (e.g., Anderson et al. 1987), flux data were grouped by depth into ≈1.0-cm intervals. The resulting ^{137}Cs profile roughly resembled that of nearby Pinawa, Manitoba, but was apparently displaced by five or six varve years, similar to the observations made using the biostratigraphic markers (Fig. 6B).

^{210}Pb Chronology

^{226}Ra activity is commonly used to estimate naturally occurring supported ^{210}Pb in lake sediments (Oldfield and Appleby 1984a). ^{226}Ra activity was determined at about 880 and 1670 mg·cm^{-2} (accumulated dry weight), corresponding to composite varve years 1900–1904 and 1800–1804, and compared well with the total ^{210}Pb determined in the

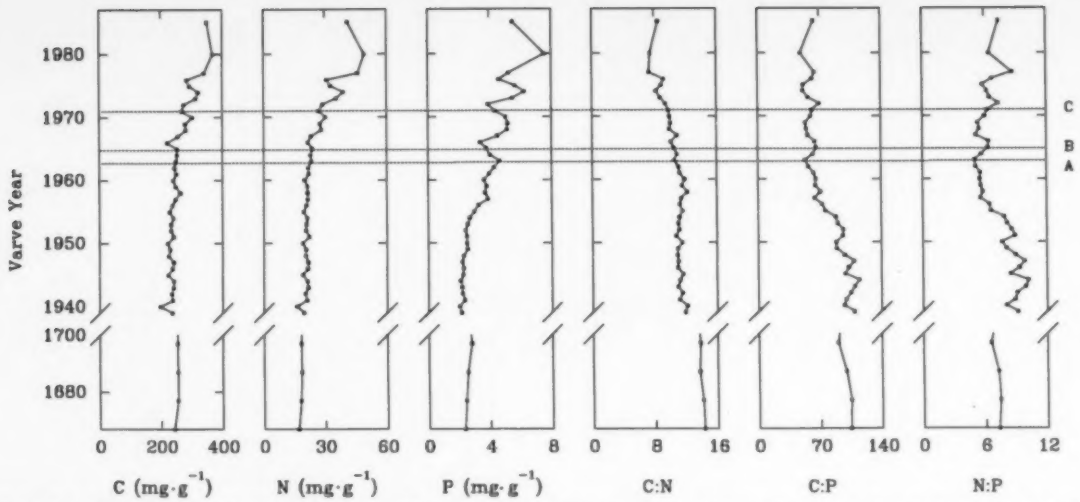

FIG. 8. Carbon (C), nitrogen (N), and phosphorus (P) dry weight concentration and ratio profiles. Vertical scale is broken to highlight recent changes corresponding to eutrophication. Biostratigraphic markers A, B, and C from varve years 1963, 1965, and 1971 represent depositional years 1969, 1971, and at least 1975, respectively.

four deepest samples (Fig. 7A). This suggested that the mean of these two ^{226}Ra values, 0.1355 Bq·g^{-1}, was probably representative of the naturally occurring supported ^{210}Pb.

In 1970, the epilimnion of Lake 227 received a tracer addition of 360 × 10^6 Bq of ^{226}Ra (Emerson and Hesslein 1973) and, therefore, estimates of natural unsupported ^{210}Pb in near surface samples were contaminated and could not be used for calculation of sediment age. Evidence of this addition in the sediments was shown by high ^{226}Ra at about 230 mg·cm^{-2} (accumulated dry weight), corresponding to composite varve year 1970–1971. Significant downward transport of ^{226}Ra in Lake 227 is known to have occurred (Anderson et al. 1987) and, therefore, ^{210}Pb resulting from decay of the ^{226}Ra spike would be expected rather deep in the core. Clearly, this process has had a marked effect on total ^{210}Pb concentrations measured in the upper five samples (Fig. 7A). Below the contaminated zone, samples analyzed for total ^{210}Pb described a log-linear relationship with accumulated dry weight indicative of relatively constant sedimentation rates (Oldfield and Appleby 1984a).

Uncontaminated ^{210}Pb samples for varve years from 1860 to 1934 generated an estimated average sediment accumulation rate that was in close agreement with the rate calculated directly from weighed sediment couplets over this same interval (Fig. 7B). Linear regression produced an average sedimentation rate of 8.7 ± 0.2 (SD) mg·cm^{-2}·yr^{-1}, measured directly from varve sediment sampling, and 6.2 ± 0.8 mg·cm^{-2}·yr^{-1} from ^{210}Pb. In addition, natural unsupported ^{210}Pb was determined to varve year 1860 (Fig. 7B), consistent with the expected 100 to 150 yr dating limit of ^{210}Pb.

Elemental Profiles

Carbon and N concentrations did not vary between varve years 1939 and 1965, but then gradually increased (Fig. 8), probably in response to fertilization. Phosphorus concentration also increased gradually, although the increase began near varve year 1950, several years prior to artificial P additions. A notable absence of distinct changes in either the nutrient concentration profiles or in elemental ratios (Fig. 8), was at least partly a result of sampling difficulties. It was virtually impossible to completely separate couplets, so the profiles may be unintentionally smoothed. Therefore, it is difficult to use this data set for assessment of varve chronology.

Discussion

Reliability of Varve Chronology

Qualitative analysis of lamination content indicated that the prefertilization couplets represented varves. However, independent biostratigraphic markers and radiochemical and geochemical chronologies consistently suggested that the postfertilization couplets were more complex. Errors occurred as a result of assigning varve years by simply counting couplets back from the surface of the core.

Known major siliceous and nonsiliceous algal blooms from archived phytoplankton records corresponded to distinct laminations in the algal record of the core and established independent postfertilization dates that differed from the varve year estimates. The algal taxa indicative of these events and found in the sediments included (1) the *Synedra* blooms of 1971, 1977, and 1982 (2) the remains of non-N_2-fixing filamentous cyanobacteria (*Oscillatoria*) and green algae (*Staurastrum* and *Scenedesmus*) dominant in the plankton from 1969–1974 and suggestive of high N:P ratio, and (3) the akinetes of *Aphanizomenon* and *Anabaena*, representative of a low N:P ratio and N_2-fixation beginning in 1975. Thin laminae made up of various chrysophyte cysts and scales, found in conjunction with the more dramatic events mentioned above, correlated with the phytoplankton record and supplied additional corrobative evidence for the postfertilization chronology (H.J. Kling, unpublished data). These observations suggest that the original varve-year estimates of 1963, 1965, and 1971 more accurately represented 1969, 1971, and at least 1975, respectively. Such corrections were consistent with the ^{137}Cs chronology of ≈1 cm slices.

At least two explanations may, together, be responsible for the incorrect old varve chronology. First, more than one couplet per year may have been deposited after the 1969

fertilization of the lake. Following the onset of fertilization, the phytoplankton archival records indicated that the regular sequence of chrysophyte cyst and diatom deposition became chaotic. In 1970 there were three chrysophyte pulses in the plankton record, and in 1971 there were four peaks in addition to the diatom bloom, whereas in 1972 there was only one chrysophyte peak (Findlay and Kling 1975). Virtually no chrysophyte pulses occurred in 1974, but three major blooms were recorded in 1975 (Findlay 1978). Several couplets in the post-1969 strata contained several light laminations per year, and it is not yet totally clear how to count them. Further analysis of sediment cores from Lake 227 are required to provide an in-depth assessment of these multiple events and their relationship to sediment chronology.

Some of the chronological error undoubtedly resulted from misidentification of varves younger than 1977, where laminations were indistinct. Close correlation between algal standing crop, fossil pigments, and zooplankton from a similar core taken in 1990 suggest that this error arose in sediments deposited after the historical biomass maximum in 1981 (P.R. Leavitt, University of Regina, Regina, Sask., personal communication).

Although independent assignment of years by ^{210}Pb dating was not possible because of ^{226}Ra experiments in 1970 (Emerson and Hesslein 1973), the ^{210}Pb and varve chronology for pre-1940 sediments gave similar estimates of sedimentation rates and suggest that cumulative errors in estimating sediment age by varve count were minimal, but only when long chronologies were compared. Similarly, comparison of bulk sediment accumulation and organic content profiles with those from another core (Leavitt and Findlay 1994; Fig. 9) also confirmed the reliability of the long-term varve chronology. Despite different sampling methods and resolution, sediment accumulation rate and loss-on-ignition trends were quite similar, including both a minimum near varve year 1930 and a general decline in organic content in varve years from 1850 to 1930.

In summary, we emphasize that all of the chronologies have some error component and none should be accepted as the absolute truth. Despite inherent difficulties in subsampling millimetre-thick strata and analytical limitations (e.g., very small sample size for ^{137}Cs), several independent chronologies were relatively consistent with each other.

Implications for Studies of Laminated Sediments

Unambiguous establishment of the annual nature of biogenic laminations requires demonstration that patterns of deposition correspond to seasonal production of material such as plankton, zooplankton, and organic detritus. Sediments with alternating light and dark laminations are too often assumed to be annual couplets, although ^{137}Cs and ^{210}Pb have been used to demonstrate the achronology of some sediments (Smith 1978; Stihler et al. 1992; Crusius and Anderson 1992). In some cases, ^{210}Pb and ^{137}Cs have been used to confirm the annual nature of sedimentary couplets (Koide et al. 1972; Livingstone and Cambray 1978; Appleby et al. 1979; Brunskill et al. 1984; Erten et al. 1985; Brunskill and Ludlam 1988). These studies are used as evidence for a relatively constant atmospheric ^{210}Pb flux to nonlaminated sediments, but complex models of sediment mixing and pore-water diffusion are necessary to unravel chronological details. Varved lake sediments have been used to correct and calibrate ^{14}C activity versus time (Geyh et al. 1970, 1971; Lotter 1991). A few papers have revealed apparent inconsistencies between ^{210}Pb and ^{137}Cs, and other independent time markers (Davis et al. 1984; Bloesch and Evans 1982), but generally there has been good success at relating these radiochemical tools to pollen and magnetic stratigraphy and particle mixing (Smith et al. 1986–1987; Bruland et al. 1975; Koide et al. 1975; Brush et al. 1982; Oldfield and Appleby 1984b; Robbins 1986). Our results confirmed the utility of ^{210}Pb in the estimation of mass accumulation rates, but indicated that ^{137}Cs deposition in profundal sediment of this lake was curiously episodic, and this could not be explained by pore-water diffusion.

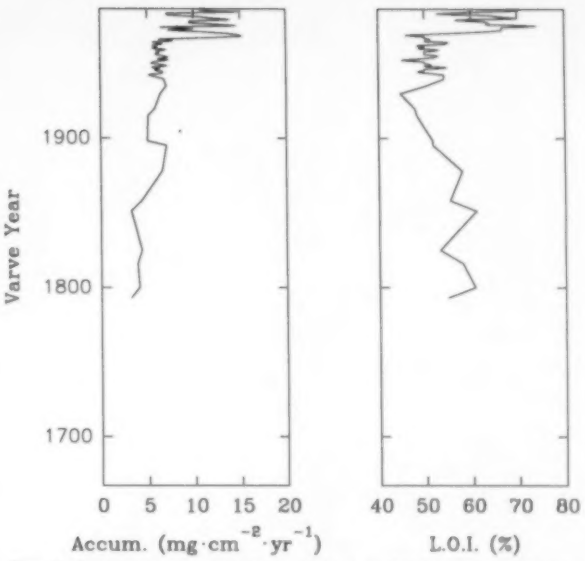

FIG. 9. Sediment accumulation and loss-on-ignition (L.O.I.) profiles from Leavitt and Findlay (1994). Data plotted on scales similar to Fig. 2 to facilitate comparison.

Simple varve counting from the modern sediment–water interface may be acceptable in a validated undisturbed system, but our study showed that the reliability of such counts may decrease after nutrient supply changes dramatically. True varves were present throughout the Lake 227 core, but subannual and indistinct laminations in surface sediments hampered establishment of an accurate time scale. Fortunately, the use of multiple chronologies derived from radioisotopes, geochemistry, and biological remains were instrumental in recognizing these problems and setting the stage for further study.

Acknowledgments

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References

ANDERSON, R.F., S.L. SCHIFF, AND R.H. HESSLEIN. 1987. Determining sediment accumulation and mixing rates using ^{210}Pb, ^{137}Cs, and other tracers: problems due to postdepositional mobility or coring artifacts. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 231–250.

APPLEBY, P.G., F. OLDFIELD, R. THOMPSON, P. HUTTENEN, AND K. TOLONEN. 1979. ^{210}Pb dating of annually laminated lake sediments from Finland. Nature (Lond.) 280: 53–55.

ARMSTRONG, F.A.J., AND D.W. SCHINDLER. 1971. Preliminary chemical characterization of waters in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 171–187.

BATES, R.L., AND J.A. JACKSON. 1987. Glossary of geology. American Geological Institute, Alexandria, Va. 778 p.

BLOESCH, J., AND R.D. EVANS. 1982. ^{210}Pb dating of sediments compared with accumulation rates estimated by natural markers and measured with sediment traps. Hydrobiologia 92: 579–586.

BRULAND, K.W., M. KOIDE, C. BOWSER, L.J. MAHER, AND E.D. GOLDBERG. 1975. ^{210}Pb and pollen geochronologies on Lake Superior sediments. Quat. Res. 5: 89–98.

BRUNSKILL, G.J., AND S.D. LUDLAM. 1988. The variation of annual ^{210}Pb flux to varved sediments of Fayetteville Green Lake, N.Y., from 1885 to 1965. Verh. Int. Ver. Theor. Angew. Limnol. 23: 848–854.

BRUNSKILL, G.J., S.D. LUDLAM, AND T.-H. PENG. 1984. Fayetteville Green Lake, New York, USA. VIII. Mass balance for ^{137}Cs in water, varved and non-varved sediments. Chem. Geol. 44: 101–117.

BRUNSKILL, G.J., D. POVOLEDO, B.W. GRAHAM, AND M.P. STAINTON. 1971. Chemistry of surface sediments of sixteen lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 277–294.

BRUNSKILL, G.J., AND D.W. SCHINDLER. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139–155.

BRUSH, G.S., E.A. MARTIN, R.S. DEFRIES, AND C.A. RICE. 1982. Comparisons of ^{210}Pb and pollen methods for determining rates of estuarine sediment accumulation. Quat. Res. 18: 196–217.

CRUSIUS, J., AND R.F. ANDERSON. 1992. Inconsistencies in accumulation rates of Black Sea sediments inferred from records of laminae and ^{210}Pb. Paleoceanography 7: 215–227.

DAVIDSON, G.A. 1988. A modified tape-peel technique for preparing permanent qualitative microfossil slides. J. Paleolimnol. 1: 229–234.

DAVIS, R.B., C.T. HESS, S.A. NORTON, D.W. HANSON, K.D. HOAGLAND, AND D.S. ANDERSON. 1984. ^{137}Cs and ^{210}Pb dating of sediments from soft water lakes in New England and Scandinavia, a failure of ^{137}Cs dating. Chem. Geol. 44: 151–185.

DEAN, W.E., J.P. BRADBURY, R.Y. ANDERSON, AND C.W. BARNOSKY. 1984. The variability of Holocene climate change: evidence from varved lake sediments. Science (Wash., D.C.) 226: 1191–1194.

EMERSON, S., AND R. HESSLEIN. 1973. Distribution and uptake of artificially introduced radium-226 in a small lake. J. Fish. Res. Board Can. 30: 1485–1490.

ERTEN, H.N., H.R. VON GUNTEN, E. ROSSLER, AND M. STURM. 1985. Dating of sediments from Lake Zurich with ^{210}Pb and ^{137}Cs. Schweiz. Z. Hydrol. 47: 5–11.

FINDLAY, D.L. 1978. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1974 to 1976. Can. Fish. Mar. Serv. MS Rep. 1466: 41 p.

FINDLAY, D.L. 1981. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1977 to 1979. Can. MS Rep. Fish. Aquat. Sci. 1627: 40 p.

FINDLAY, D.L. 1983. Seasonal successions of phytoplankton in five lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1980 to 1982. Can. MS Rep. Fish. Aquat. Sci. 1710: 30 p.

FINDLAY, D.L., AND H.J. KLING. 1975. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario following artificial eutrophication. Can. Fish. Mar. Serv. Tech. Rep. 513: 53 p.

GEYH, M.A., J. MERKT, AND H. MULLER. 1970. ^{14}C datierung limnischer Sedimente und die Eichung der ^{14}C-Zeitskala. Naturwissenschaften 57: 564–567.

GEYH, M.A., J. MERKT, AND H. MULLER. 1971. Sediment-, Pollen-, und Isotopenanalysen an jahreszeitlich geschichteten Ablangerungen im zentralen Teil des Schleinsees. Arch. Hydrobiol. 69: 366–399.

HUTTUNEN, P., AND J. MERILÄINEN. 1978. New freezing device providing large unmixed sediment samples from lakes. Ann. Bot. Fenn. 15: 128–130.

KLING H.J., AND S.K. HOLMGREN. 1972. Species composition and seasonal distribution of phytoplankton in the Experimental Lakes Area, Northwestern Ontario. Fish. Res. Board Can. Tech. Rep. 337: 51 p.

KOIDE, M., J.J. GRIFFIN, AND E.D. GOLDBERG. 1975. Records of plutonium fallout in marine and terrestrial samples. J. Geophys. Res. 80: 4153–4162.

KOIDE, M., A. SOUTAR, AND E.D. GOLDBERG. 1972. Marine geochronology with ^{210}Pb. Earth Planet. Sci. Lett. 14: 442–446.

LEAVITT, P.R., AND D.L. FINDLAY. 1994. Calibration of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci. 51: 2286–2299.

LIVINGSTONE, D., AND R.S. CAMBRAY. 1978. Confirmation of ^{137}Cs dating by algal stratigraphy in Rostherne Mere. Nature (Lond.) 276: 259–261.

LOTTER, A.F. 1991. Absolute dating of the Late Glacial Period in Switzerland using annually laminated sediments. Quat. Res. 35: 321–330.

NATIONAL RESEARCH COUNCIL OF CANADA (NRCC). 1983. Radioactivity in the Canadian aquatic environment. NRCC 19250: 292 p.

OLDFIELD, F., AND P.G. APPLEBY. 1984a. Empirical testing of ^{210}Pb dating models for lake sediments, p. 93–124. *In* E.Y. HAWORTH AND J.W.G. LUND [ed.] Lake sediments and environmental history. University of Minnesota Press, Minneapolis, Minn.

OLDFIELD, F., AND P.G. APPLEBY. 1984b. A combined radiometric and mineral magnetic approach to recent geochronology in lakes affected by catchment disturbance and sediment redistribution. Chem. Geol. 44: 67–83.

O'SULLIVAN, P.E. 1983. Annually-laminated lake sediments and the study of Quaternary environmental changes—a review. Quat. Sci. Rev. 1: 245–313.

PERKINS, J.A., AND J.D. SIMS. 1983. Correlation of Alaskan varve thickness with climatic parameters, and use in paleoclimatic reconstruction. Quat. Res. 20: 308–321.

RENBERG, I., U. SEGERSTROM, AND J.-E. WALLIN. 1984. Climatic reflection in varved lake sediments, p. 249–256. *In* N.-A. MORNER AND W. KARLEN [ed.] Climatic changes on a yearly to millennial basis. D. Reidel Publishing Company, Dordrecht, The Netherlands.

RITCHIE, J.C., AND J.R. MCHENRY. 1990. Application of radioactive fallout cesium-137 for measuring soil erosion and sediment accumulation rates and patterns: a review. J. Environ. Qual. 19: 215–233.

ROBBINS, J.A. 1978. Geochemical and geophysical applications of radioactive lead, p. 285–393. *In* J.O. NRIAGU [ed.] The biogeochemistry of lead in the environment. Elsevier/North Holland, Biomedical Press, Amsterdam.

ROBBINS, J.A. 1986. A model for particle-selective transport of tracers in sediment with conveyor belt deposit feeders. J. Geophys. Res. 91(C7): 8542–8558.

ROBBINS, J.A., AND D.N. EDGINGTON. 1975. Determination of recent sedimentation rates in Lake Michigan using lead-210 and cesium-137. Geochim. Cosmochim. Acta 39: 285–304.

ROBBINS, J.A., D.N. EDGINGTON, AND A.L.W. KEMP. 1978. Comparative ^{210}Pb, ^{137}Cs, and pollen geochronologies of sediments from Lakes Ontario and Erie. Quat. Res. 10: 256–278.

SAARNISTO, M. 1986. Annually laminated lake sediments, p. 343–370. *In* B.E. BERGLUND [ed.] Handbook of Holocene palaeoecology and palaeohydrology. John Wiley & Sons, New York, N.Y.

SCHINDLER, D.W. 1971. Light, temperature, and oxygen regimes of selected lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 157–169.

SCHINDLER, D.W., F.A.J. ARMSTRONG, S.K. HOLMGREN, AND G.J. BRUNSKILL. 1971. Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by addition of phosphate and nitrate. J. Fish. Res. Board Can. 28: 1763–1782.

SCHINDLER, D.W., R.H. HESSLEIN, AND M.A. TURNER. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 26–33.

SCHINDLER, D.W., H. KLING, R.V. SCHMIDT, J. PROKOPOWICH, V.E. FROST, R.A. REID, AND M. CAPEL. 1973. Eutrophication of Lake 227 by addition of phosphorus and nitrate: the second, third, and fourth years of enrichment, 1970, 1971, and 1972. J. Fish. Res. Board Can. 30: 1415–1440.

SIMOLA, H. 1977. Diatom succession in the formation of annually laminated sediment in Lovojarvi, a small eutrophicated lake. Ann. Bot. Fenn. 14: 143–148.

SMITH, J.N., B.P. BOUDREAU, AND V. NOSHKIN. 1986–1987. Plutonium and ^{210}Pb distributions in northeast Atlantic sediments: subsurface anomalies caused by non-local mixing. Earth Planet. Sci. Lett. 81: 15–28.

SMITH, N.D. 1978. Sedimentation processes and patterns in a glacier-fed lake with low sediment input. Can. J. Earth Sci. 15: 741–756.

STAINTON, M.P., M.J. CAPEL, AND F.A.J. ARMSTRONG. 1977. The chemical analysis of fresh water. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

STIHLER, S.D., D.B. STONE, AND J.E. BEGET. 1992. "Varve" counting vs. tephrochronology and ^{137}Cs and ^{210}Pb dating: a comparative test at Skilak Lake, Alaska. Geology 20: 1019–1022.

TORGENSON, T., AND M.E. LONGMORE. 1984. ^{137}Cs diffusion in highly organic sediment of Hidden Lake, Fraser Island, Queensland. Aust. J. Mar. Freshwater Res. 35: 537–548.

WILKINSON, P. 1985. The determination of environmental levels of uranium and thorium series isotopes and ^{137}Cs in aquatic and terrestrial samples. Can. Spec. Publ. Fish. Aquat. Sci. 78: 51 p.

Comparison of Fossil Pigments with 20 Years of Phytoplankton Data from Eutrophic Lake 227, Experimental Lakes Area, Ontario[1]

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Leavitt, P.R., and D.L. Findlay. 1994. Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci. 51: 2286–2299.

Fossil pigments from annually laminated sediments were calibrated with coeval phytoplankton data (1970–1989) from experimentally eutrophied Lake 227 in the Experimental Lakes Area, Ontario. Concentrations of ubiquitous pigments (β-carotene, pheophytin *a*) were correlated to total algal biomass standing crop ($r = 0.56–0.65$; $P < 0.01$) during the ice-free seasons, but not to carbon fixation or water-column chlorophyll (Chl). Indicator pigments were correlated to ice-free season algal biomass for cyanobacteria (echinenone, aphanizophyll) and chlorophytes (lutein–zeaxanthin, pheophytin *b*)($r = 0.53–0.55$, $P < 0.05$), weakly correlated for cryptophytes (alloxanthin, α-carotene; $r = 0.32–0.40$, $P < 0.10$), but were uncorrelated for chrysophytes and diatoms (fucoxanthin, Chl *c*) or dinoflagellates (peredinin). Premanipulation concentrations of fossil pigments (nmol pigment · (g organic matter)$^{-1}$) from green algae and filamentous cyanobacteria (myxoxanthophyll) increased 4- to 10-fold in response to eutrophication of Lake 227. N_2-fixing cyanobacteria (recorded as aphanizophyll) replaced chlorophytes after the nitrogen additions decreased threefold in 1975. In contrast, accumulation rates of pigments (nmol pigment · m^{-2} · yr^{-1}) were rarely correlated with algal standing crop or production and were less satisfactory than fossil concentrations for the purpose of detecting changes in phytoplankton community composition.

L'ancienneté de pigments fossiles dans des sédiments déposés en couches annuelles a été étalonnée au moyen de données contemporaines sur le phytoplancton (1970–1989) du lac 227, eutrophié expérimentalement, dans la région des lacs expérimentaux de l'Ontario. La concentration des pigments trouvés partout (β-carotène, phéophytine *a*) est en corrélation avec la biomasse algaire totale ($r = 0,56–0,65$; $P < 0,01$) durant les saisons où il n'y a pas de glace, mais pas avec la fixation du carbone ou la teneur en chlorophylle (Chl) dans la colonne d'eau. Il existe une corrélation entre les pigments indicateurs et la biomasse algaire des cyanobactéries (échinénone, aphanizophylle) et celle des chlorophytes (lutéine–zéaxanthine, phéophytine *b*)($r = 0,53–0,55$, $P < 0,05$) durant les saisons libres de glace; il existe une faible corrélation avec celle des cryptophytes (alloxanthine, α-carotène; $r = 0,32–0,40$, $P < 0,10$), mais pas de corrélation avec celles des chrysophytes et des diatomées (fucoxanthine, Chl *c*) ou des dinoflagellés (pérédinine). La concentration, mesurée avant la transformation du lac, des pigments fossiles (nmol de pigments · (g matière organique)$^{-1}$) provenant des algues vertes et des cyanobactéries filamenteuses (mixoxanthophylles), s'est accrue de 4 à 10 ordres de grandeur en réponse à l'eutrophisation du lac 227. Les cyanobactéries fixatrices de N_2 (sous forme d'aphanizophylle) ont délogé les chlorophytes après que les apports d'azote aient diminué de trois ordres de grandeur en 1975. Par ailleurs, les taux d'accumulation de pigments (nmol pigment · m^{-2} · an^{-1}) étaient rarement en corrélation avec la production ou la biomasse algaire et donnaient des résultats moins satisfaisants que les concentrations fossiles pour ce qui était de détecter des variations dans la composition de la communauté phytoplanctonique.

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Long-term historical records document environmental change and provide the framework for evaluating ecosystem disturbance by humans. However, reliable annual records are rare because of the high costs and changes in methodology, and are often not initiated until after environmental change is underway. In many lakes, paleolimnology is the sole source of historical information.

Fossil pigments are promising paleoecological indicators that can discriminate between most major algal groups. Chlorophylls (Chl) and carotenoids have been used to detect changes in phytoplankton composition (Ridout and Morris 1985; Foy 1987; Leavitt and Carpenter 1990a), algal sedimentation (Welschmeyer and Lorenzen 1985a, 1985b; Carpenter et al. 1986, 1988; Leavitt and Carpenter 1990a), and postglacial changes in primary production (Brown 1969; Sanger 1988). When plant pigments are used in conjunction with

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zooplankton microfossil analyses, paleolimnologists can reconstruct predator–prey interactions in whole-lake food-web manipulations (Leavitt et al. 1989, 1993). However, mass-balance budgets show that sediments are also biased towards pigments that are incorporated into rapidly sinking material (herbivore feces, large colonies, consolidated detritus) (Welschmeyer and Lorenzen 1985a, 1985b; Carpenter et al. 1986, 1988). Although geographic surveys show that gross fossil pigment concentration is proportional to total algal abundance (Gorham et al. 1974; Flanney et al. 1982; Guilizzoni et al. 1983; Brenner and Binford 1988), individual fossil pigments have yet to be quantitatively compared with long-term, continuous phytoplankton data.

We used 20 yr of phytoplankton data from a small, experimentally eutrophied lake to determine if fossil pigments accurately record changes in gross algal community composition. Our objectives were: (1) to determine which aspects of phytoplankton abundance (carbon (C) fixation, biomass, Chl) are recorded by fossil pigments; (2) to establish if fossil pigments differ in their reliability as indicators of a given algal group; (3) to compare fossil pigment concentration and accumulation rate as measures of algal abundance; (4) to determine if fossil pigments record planktonic events with annual resolution; and (5) to compare pre- and post-fertilization lake conditions using fossil pigment analyses.

Lake 227 is ideal for high-resolution paleolimnology. The water column stratifies quickly and strongly in the summer (thermocline = 2–4 m), the hypolimnion is anoxic for much of the year, and complete water column mixing is rare (Schindler 1971). Sediments are annually laminated (varved) throughout the last 400 yr, although recent sediments also contain distinct subannual laminae (Wolfe et al. 1994). The combination of a cold, dark, anoxic depositional environment, undisturbed sediments, and large changes in phytoplankton community composition maximizes the opportunity for formation of a reliable fossil record.

Site Description

Lake 227 has been experimentally eutrophied since 1969 when the natural flux of phosphorus (P, as Na_2HPO_4 or H_3PO_4) was increased 10-fold and nitrogen (N, as $NaNO_3$) was added in an N:P ratio of 13:1 by mass. Subsequent rapid algal growth established that eutrophication was not C limited (Schindler et al. 1971, 1973). In 1975, the mass ratio of N:P was decreased from 13:1 to 5:1 to determine if the imposed N deficit could be alleviated through fixation of atmospheric N_2 by cyanobacteria (Schindler 1977, 1987). The general history of the manipulation was summarized by Schindler (1987). Below, we briefly review site characteristics and premanipulation food-web composition.

Lake 227 is a small (5 ha), relatively deep (Z_{max} = 10 m, Z_m = 4.4 m), dilute headwater lake situated in the Experimental Lakes Area (ELA) of northwestern Ontario (49°2′N, 93°42′W). Regional geology and soils are described by Brunskill and Schindler (1973). Local forests include jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), with some poplar (*Populus tremuloides*) and birch (*Betula papyrifera*). The understory is mostly composed of juniper (*Juniperus communis*). Evidence from fire scars suggests that the forest in the vicinity of Lake 227 last burned in 1870–1880 (D. Schindler, Department of Zoology, University of Alberta, Edmonton, Alta., unpublished data). Fire in 1980 burned a small portion of the watershed (<2%), but the local forests are otherwise undisturbed.

The premanipulation algal community was characteristic of stained (color = 18–20 Pt units; Secchi depth = 1.3–2.6 m), unproductive (Chl = 3–18 $\mu g \cdot L^{-1}$) waters and consisted mainly of chrysophytes (Schindler et al. 1973; Findlay and Kling 1975), spring populations of cryptophytes (Kling and Holmgren 1972), and littoral diatoms (Stockner 1971; Stockner and Armstrong 1971). Summer algal standing stocks were estimated to be 1–3 $g \cdot m^{-3}$ (Kling and Holmgren 1972; Findlay and Kling 1975). Zooplankton were mostly small-bodied crustaceans and included *Bosmina longirostris*, *Diaptomus minutus*, and *Tropocyclops prasinus mexicanus* (Patalas 1971; Malley et al. 1988; Hann et al. 1994). The fish community of Lake 227 is simple and composed of planktivorous fishes: fathead minnows (*Pimephales promelas*), pearl dace (*Margariscus margarita*), northern redbelly dace (*Phoxinus eos*), and finescale dace (*P. neogaeus*) (Beamish et al. 1976).

Methods

Plankton Samples

Water samples for determination of phytoplankton composition and primary production were collected approximately biweekly during the ice-free period (Findlay and Kasian 1987). Between 1969 and 1975, discrete samples were retrieved from five to seven depths using a 2-L van Dorn water bottle. An integrating sampler was used after 1974 to obtain samples from the epilimnion, metalimnion, or hypolimnion (Shearer 1978). Strata were defined by light and temperature. Samples were preserved with Lugol's iodine solution, settled, and enumerated using the Utermohl technique (Nauwerck 1963; Kling and Holmgren 1972; Findlay and Kasian 1987). Wet weight biomass values were estimated from geometric approximations of cell shape and estimated cell density (Vollenweider 1968; Findlay and Kasian 1987). Algal species were grouped according to pigment composition to allow direct comparison of phytoplankton abundance and concentrations of fossil pigments.

Carbon fixation (phytoplankton primary production) was measured using in situ bottle incubations with ^{14}C during 1969–1972. This technique was discontinued in 1973 in favor of the incubator–numerical model of Fee (1973, 1980). The evolution of techniques for sampling, sample incubation, scintillation counting, and numerical integration has necessitated the conversion of older C-fixation data to 1982 equivalents, as discussed by Shearer et al. (1985, 1987). Consequently, data from 1973–1981 were reduced by 1.22-fold (E. DeBruyn, Department of Fisheries and Oceans, Winnipeg, Man., personal communication). Estimates of C fixation obtained by in situ bottle incubations could not be converted and were discarded. Annual C fixation was expressed as morphometry-corrected, mean areal C uptake under actual weather conditions (mg C incorporated $\cdot m^{-2}$).

Chlorophyll *a* standing crops were estimated from discrete samples collected following Stainton et al. (1977) and Shearer et al. (1987). Prior to 1981, particles were filtered onto Whatman GF/C filters and extracted by grinding for 1 min in 90% acetone. Extracts were refiltered before quantification of total chlorophyllous pigments by calibrated fluorometry (Shearer et al. 1987). In subsequent years, Chl was extracted by allowing filters to stand overnight in the

dark at 4°C in 95% methanol (Shearer et al. 1987). Average areal Chl standing crops (mg Chl · m^{-2}) were calculated for each year and corrected for morphometry (Fee 1980).

Sediment Samples

Sediments were sampled from the deepest portion of Lake 227 in January 1990 using a freeze corer (Swain 1973). Frozen cores were prepared for fine-interval sectioning by cutting the core lengthwise into four sections, removing the outermost 5 mm of sediment, and freeze-drying the cleaned core sections for 24 h (Leavitt et al. 1989). Recent sediments (ca. 1945–1990) were examined for the presence of annual cycles of deposition (varves) using sediment color, texture, and coherence (resistance to shear stress with a pin). Groups of laminae were tentatively identified to year of deposition and isolated manually (1945–1990) at −8°C within 3 h of lyophilization. Sediments deposited after ca. 1970 contained distinct subannual laminae (Wolfe et al. 1994) and algal mat intrusions that may have obscured annual cycles of sediment deposition. Older sediments (1945–1970) were regularly laminated and were sampled in 5-yr increments at ca. 15-yr intervals. Samples were relyophilized for 2 h and stored under an N_2 atmosphere, in the dark, at −20°C for 24 h before further analysis (Leavitt et al. 1989).

Aliquots of annual samples were analyzed for siliceous algal microfossils (Zeeb et al. 1994), invertebrate remains (Hann et al. 1994), and plant pigments (this report). An earlier core was analyzed for elemental composition, geochronology (^{210}Pb, ^{137}Cs), and laminae fine structure (Wolfe et al. 1994).

Sedimentary pigments were extracted, filtered, and dried under N_2 gas following the procedures of Leavitt et al. (1989). Carotenoid, Chl, and pigment-derivative concentrations were quantified using a Waters HPLC system following the reversed-phase procedure of Mantoura and Llewellyn (1983), as modified by Leavitt et al. (1989). An internal reference standard (3.2 mg · L^{-1}) of Sudan II (Sigma Chemical Corp., St. Louis, Mo.) was injected into each sample. This dye runs at a central, unique position on the chromatogram (near myxoxanthophyll), has carotenoid-like absorption characteristics (λ max = 485, 442.5 nm in acetone), and allows correction for dilution and injection errors. If the reference peak area was different from expectations based on prior calibration, a percent deviation was calculated and used to correct all pigment peak areas. Reference peaks were typically within 10% of expectations.

Pigments isolated from sediments were compared with those from unialgal cultures (Leavitt et al. 1989). Spectral characteristics, chromatographic mobility, and functional group assays (Liaaen-Jensen 1971) were used to identify pigments from all sources (Leavitt et al. 1989). Acid and methyl derivatives of chlorophyllous pigments were created either by aqueous–alcohol extraction (chlorophyllides) or by acidification following the procedures of Leavitt et al. (1989). Pyropheophytin *a* and a compound tentatively identified as pyropheophorbide *a* were collected by HPLC isolation from sediments of several lakes. Not all fossil pigments were positively identified. We restrict our analysis to carotenoids characteristic of cryptophytes (alloxanthin, α-carotene in part), diatoms and chrysophytes (fucoxanthin), chlorophytes and cyanobacteria (lutein–zeaxanthin), all cyanobacteria (echinenone), filamentous or colonial cyanobacteria (myxoxanthophyll), and N_2-fixing cyanobacteria (aphanizophyll), as well as the major *a*, *b*, and *c*-phorbins. Lutein from green algae and zeaxanthin from cyanobacteria coelute on our HPLC system. Similarly, chromatographic peaks from aphanizophyll (*Aphanizomenon*), oscillaxanthin (Oscillatoriaceae), and 4-keto-myxoxanthophyll (*Anabaena*) were not completely resolved on our system, and are presented as aphanizophyll.

Bulk sediment and pigment accumulation rates were estimated from partly freeze-dried core sections. Bulk accumulation rates (g dry sediment · cm^{-2} · yr^{-1}) were calculated as the mass of sediments accumulated beneath a known surface area (3–5 cm^2) for each varve. Estimates of sediment accumulation were similar to those obtained from an independent analysis of varve thickness (see Wolfe et al. 1994). Organic content of varves was estimated by weight loss on ignition for 1 h at 500°C (Dean 1974). Pigment concentration was expressed as nmol pigment · (g organic matter)$^{-1}$ and fossil accumulation rate (nmol pigment · cm^{-2} · yr^{-1}) was calculated as the product of pigment concentration, bulk accumulation rate, and percent organic matter.

Results

Phytoplankton Response to Fertilization

Green algae (*Spondylosium planum*, *Staurastrum paradoxum*, *Staurastrum* sp.) replaced chrysophytes as the midsummer dominants, and algal biomass increased from 1–3 g · m^{-3} to 4.8 g · m^{-3} during 1969, the first year of fertilization (Schindler et al. 1971, 1973; Findlay and Kling 1975). Biomass declined during late summer as chrysophyte abundance increased, but remained above premanipulation values until mid-November when cryptophytes (*Rhodomonas minuta*, *Cryptomonas* sp., *C. pusilla*, *Katablepharis ovalis*) were the most abundant taxa (Findlay and Kling 1975). These algae remained important until fertilization resumed in May 1970, when chrysophytes again increased.

Green algae and cyanobacteria replaced chrysophytes and cryptophytes during spring fertilization in each year between 1970 and 1974 (Fig. 1; Findlay and Kling 1975). However, the chlorophyte species composition was complex and changed from year to year, with *Staurastrum* spp. and *Spondylosium* sp. common in 1970, *Chlamydomonas* sp. common in 1971, and mixtures of *Scenedesmus denticulatus*, *Ankistrodesmus falcatus* var. *spiralis*, *Oocystis submarina* var. *variabilis*, and others present after 1972 (Findlay and Kling 1975; Findlay 1978). Filamentous cyanobacteria (especially *Oscillatoria redekei*) were also generally present in the June algal community during 1970–1974 and achieved maximal abundance during late summer or fall. Midsummer biomass was typically ca. 10 g · m^{-3}, except during intense blooms when it could exceed 60 g · m^{-3}. Chrysophytes were again the most abundant algal group in the fall of 1970 and 1971, but not during 1972–1974 (Findlay and Kling 1975; Findlay 1978).

Reduced N additions in 1975 dramatically affected algal community composition (Fig. 1). Ratios of N:P declined from 13:1 to 5:1 and chrysophytes replaced chlorophytes as the most abundant algae by late July (Findlay 1978). However, hypolimnetic populations of N_2-fixing cyanobacteria *Aphanizomenon gracile* were common in August and this species, along with *Oscillatoria redekei*, dominated the

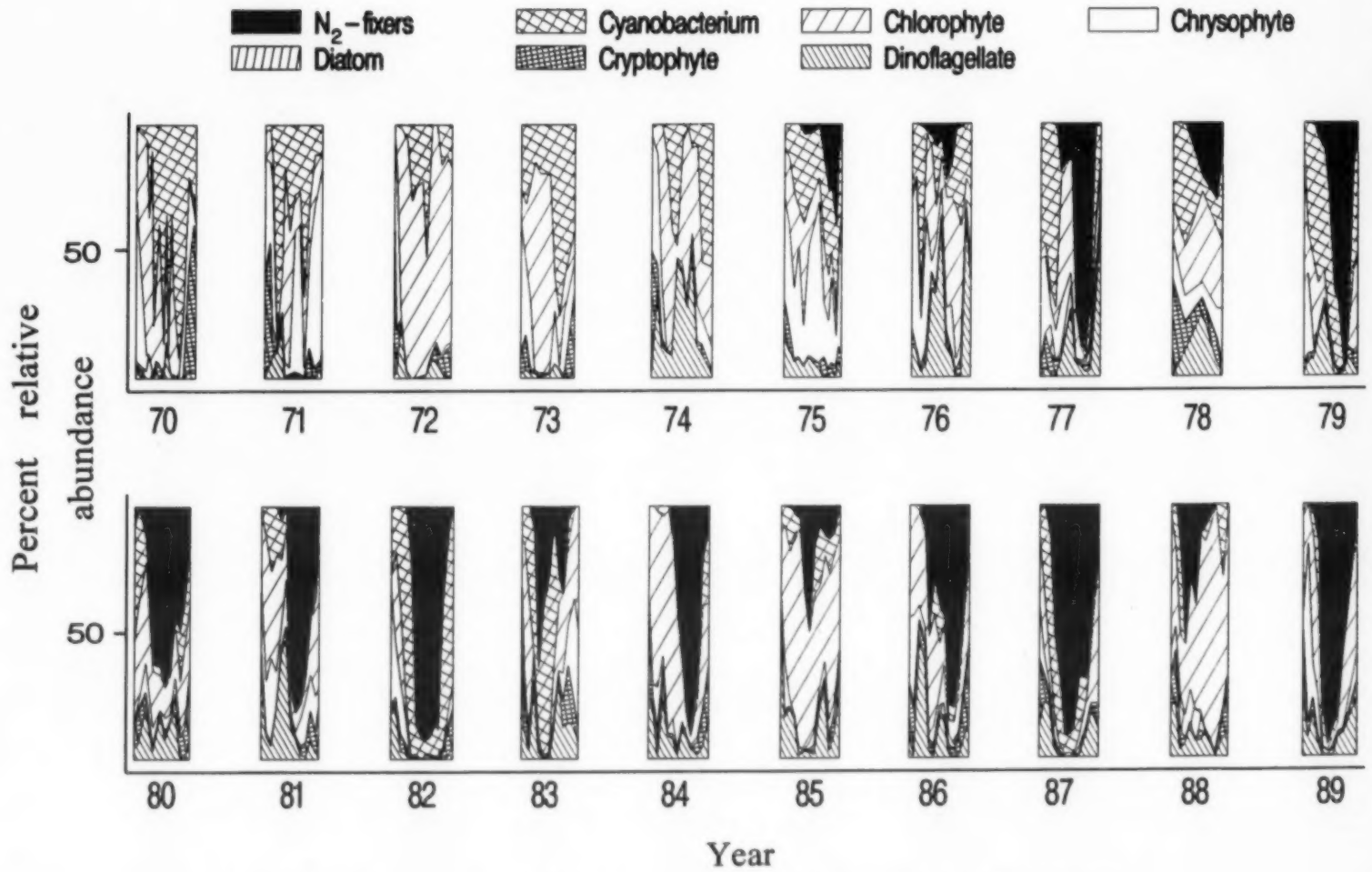

FIG. 1. Biweekly algal composition (% relative abundance) during ice-free seasons in Lake 227, 1970–1989.

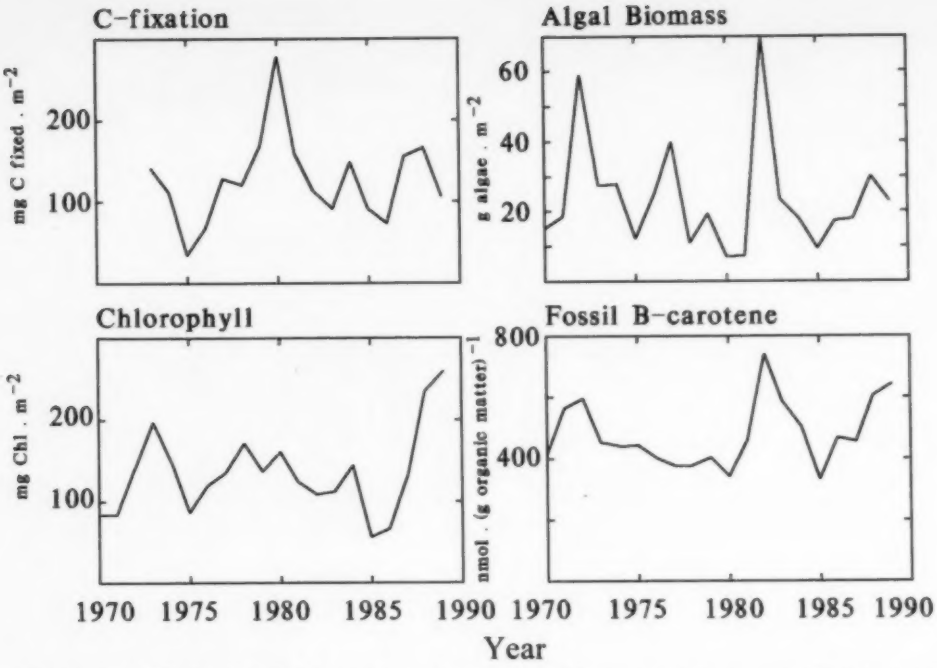

FIG. 2. Average algal carbon (^{14}C)-fixation, algal standing crop (as wet weight biomass), and fluorometrically derived chlorophyll (Chl) standing stock during ice-free seasons, and annual concentration of fossil β-carotene in varved sediments of Lake 227, 1970–1989. C-fixation data were not available for 1969–1972.

entire water column during September and October (Findlay 1978). Species succession in spring 1976 was similar to that in 1975, with chlorophytes following early blooms of chrysophytes and cryptophytes. Cyanobacteria were again important in late summer 1976, although N_2-fixing *A. gracile* populations had a lower biomass than in 1975. In contrast to previous years, dinoflagellates (Peredineae) were briefly important in late July.

Filamentous and N_2-fixing cyanobacteria continued to alternate with chlorophytes as the midsummer dominants during the 1980s. In each year, cyanobacteria replaced spring populations of chrysophytes and cryptophytes (Fig. 1; Findlay 1981). Although total algal biomass was relatively constant after 1982, the relative proportion of cyanobacteria and chlorophytes was variable. For example, the relative abundance of cyanobacteria varied from <25% of total algal biomass in 1985 and 1988 to >75% in 1987 and 1989. Cyanobacterial species composition also changed through time. *Aphanizomenon gracile* was the main N_2-fixing cyanobacteria during 1975 and 1976, but was replaced by *Anabaena flos-aquae* (now identified as *Aphanizomenon schindleri*; Kling et al. 1994) after 1976. *Aphanizomenon schindleri* and other cyanobacteria were the most important algal taxa during 1982, the historical biomass maximum for Lake 227 (Fig. 1; Findlay et al. 1994).

Carbon fixation (primary production) probably increased with fertilization, but was greatest in 1979–1981 when algal standing crop was relatively low (Fig. 2; E. DeBruyn, Department of Fisheries and Oceans, Winnipeg, Man., personal communication). In contrast, the historical production minimum occurred during 1975. Carbon fixation was not correlated with algal biomass (1973–1989, r = -0.15, $P > 0.5$), and was only weakly correlated with planktonic Chl (r = 0.43, $P < 0.10$). Planktonic Chl was not correlated to algal biomass (r = 0.12, $P > 0.6$).

Comparison of Phytoplankton and Fossil Records

General Pigments

Stable fossil indicators of total algal abundance were more closely related to phytoplankton standing crop than C fixation during ice-free seasons between 1970 and 1989 (Fig. 2). Total algal biomass was correlated with fossil concentration of β-carotene (r = 0.65, $P < 0.01$) and pheophytin *a* (r = 0.56, $P < 0.01$), but fossil concentrations were uncorrelated with ^{14}C fixation (r = 0.17 and 0.07, $P > 0.1$, respectively). Fossil pigments recorded biomass peaks in 1972, 1982, and the late 1980s, but not a smaller peak in 1977 (Fig. 2). In contrast, neither the C-fixation maximum during 1979–1981 nor a minimum in 1975 were recorded by fossil pigments (Fig. 2).

Unlike stable fossil pigments, labile fossil Chl *a* was uncorrelated with any measure of phytoplankton abundance, including water column Chl (r = −0.40, $P > 0.05$) and total algal biomass (r = 0.03, $P > 0.8$) between 1970 and 1989. However, Chl standing crop was correlated with most measures of total sediment accumulation, either expressed as bulk organic matter accumulation rates (r = 0.58, $P < 0.01$), or as accumulation of ubiquitous pigments such as β-carotene or pheophytin *a* (r = 0.65–0.68, $P < 0.01$).

Specific Pigments

Significant correlations were recorded between concentrations of indicator pigments and the annual ice-free biomass (mg · m^{-2}) of cyanobacteria (echinenone, aphanizophyll)

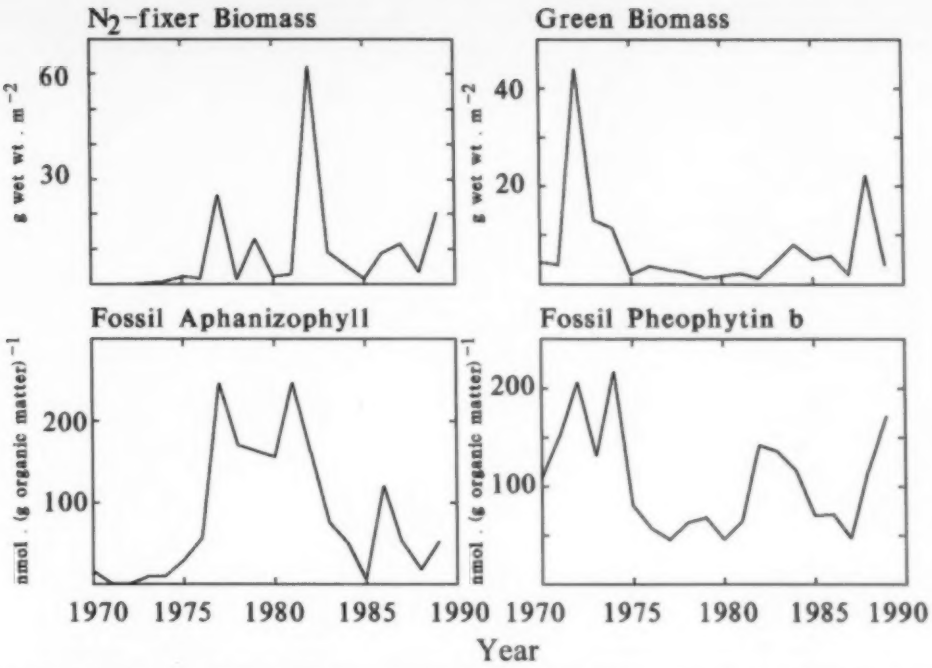

FIG. 3. Average algal standing crop during ice-free season and fossil pigment concentration in varved sediments of Lake 227 for N_2-fixing cyanobacteria (as aphanizophyll) and chlorophytes (as pheophytin *b*), 1970–1989.

and chlorophytes (pheophytin *b*, lutein-zeaxanthin) (Fig. 3, Table 1). Overall, correlations were better for more widespread pigments than for highly specific compounds. For example, rankings based on significant Pearson correlation coefficients were the inverse of those based on taxonomic specificity: β-carotene > pheophytin *a* > lutein–zeaxanthin > pheophytin *b* > echinenone > aphanizophyll (Table 1). As with ubiquitous fossils, concentrations of indicator pigments were not significantly correlated with C-fixation rates, except for a negative correlation between green algal pigments (lutein–zeaxanthin, Chl *b*) and primary production ($r = -0.46$ to -0.50, $P < 0.05$).

Some fossil pigments did not mirror changes in the biomass of their producer groups. For example, peredinin was uncorrelated to dinoflagellate abundance ($r = 0.03$, $P > 0.90$), and Chl *c* was uncorrelated to diatoms and chrysophytes ($r = 0.07$, $P > 0.75$). Both pigments degrade rapidly in the water column (Hurley and Armstrong 1991) and were usually too rare in Lake 227 sediments (<5 nmol · (g organic matter)$^{-1}$) to be reliably quantified. Similarly, alloxanthin ($r = 0.32$, $P = 0.18$) and α-carotene ($r = 0.40$, $P = 0.08$) were only weakly correlated to cryptophyte biomass, while labile fucoxanthin ($r = 0.03$, $P = 0.80$) was unrelated to chrysophyte and diatom biomass during the ice-free season.

When algal groups had multiple fossils, there was good agreement between pairs of indicator pigments (Table 2). For example, alloxanthin and α-carotene from cryptophytes were highly correlated ($r = 0.71$, $P < 0.001$), as were ubiquitous pigments β-carotene and pheophytin a ($r = 0.91$, $P < 0.001$). Weaker relationships occurred among most pigments derived from cyanobacteria (aphanizophyll, myxoxanthophyll, echinenone; $r = 0.42$–0.61, $P < 0.05$), from chlorophytes (lutein-zeaxanthin, pheophytin *b*, Chl *b*; $r = 0.54$–0.90, $P < 0.01$), and from the diatoms and chrysophytes (fucoxanthin and Chl *c*; $r = 0.47$, $P < 0.05$). Undegraded Chl *a* was not

TABLE 1. Pearson correlation coefficients between annual fossil pigment concentration (nmol · (g organic matter)$^{-1}$) or accumulation rate (nmol · cm^{-2} · yr^{-1}) and ice-free standing biomass (g · m^{-2}) of their respective algal groups ($N = 20$), 1970–1989. Chl = chlorophyll. Significance level of correlation coefficients: *$P < 0.05$; **$P < 0.01$.

Algae	Pigment	Pearson correlation coefficient (*r*)	
		Conc.	Accum.
Dinoflagellates	Peredinin	0.03	0.18
Cryptophytes	Alloxanthin	0.32	0.01
Cryptophytes	α-carotene	0.40	0.00
Cyanobacteria (total)	Echinenone	0.51*	0.28
Cyanobacteria (colonial)	Myxoxanthophyll	0.27	0.27
Cyanobacteria (N_2-fixers)	Aphanizophyll	0.45*	0.43
Diatoms + chrysophytes	Fucoxanthin	0.03	0.15
Diatoms + chrysophytes	Chl *c*	0.10	0.04
Chlorophytes	Pheophytin *b*	0.53*	0.46*
Chlorophytes	Chl *b*	0.06	0.15
Chlorophytes	Lutein–zeaxanthin	0.18	0.27
Chlorophytes + cyanobacteria	Lutein–zeaxanthin	0.55*	0.47*
All algae	β-carotene	0.65**	0.29
All algae	Pheophytin a	0.56*	0.28
All algae	Chl *a*	0.03	0.04

TABLE 2. Pearson correlation coefficient (r) between concentrations (nmol pigment · (g organic matter)$^{-1}$; above diagonal) and accumulations (nmol pigment · m^{-2} · yr^{-1}; below diagonal) of fossil pigments, 1970–1989. Significance levels: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

	L	F	A	E	M	Ap	α	β	Pa	Pb	Ca	Cb	Cc
Lutein, L	—	0.04	0.36	0.42	0.39	−0.14	0.32	0.57[b]	0.48[a]	0.66[c]	0.38	0.90[c]	0.37
Fucoxanthin, F	0.71[c]	—	0.62[b]	0.06	−0.10	−0.16	0.54[a]	0.42	0.41	0.09	0.22	0.05	0.47[a]
Alloxanthin, A	0.80[c]	0.96[c]	—	0.19	0.32	0.09	0.71[c]	0.56[b]	0.58[b]	0.32	0.20	0.29	0.38
Echinenone, E	0.75[c]	0.60[b]	0.69[c]	—	0.42	0.61[b]	0.43	0.25	−0.00	−0.16	0.83[c]	0.46[a]	0.02
Myxoxanthophyll, M	0.41	0.29	0.41	0.61[b]	—	0.52[a]	0.39	−0.05	−0.07	0.02	0.17	0.31	0.37
Aphanizophyll, Ap	0.12	0.19	0.23	0.57[b]	0.73[c]	—	0.32	−0.13	−0.18	−0.50[a]	0.32	−0.18	0.01
α-carotene, α	0.77[c]	0.91[c]	0.95[c]	0.71[c]	0.42	0.29	—	0.56[b]	0.50[a]	0.31	0.27	0.28	0.52[a]
β-carotene, β	0.82[c]	0.89[c]	0.90[c]	0.66[c]	0.19	0.11	0.91[c]	—	0.91[c]	0.64[b]	0.23	0.49[a]	0.30
Pheophytin *a*, Pa	0.78[c]	0.89[c]	0.88[c]	0.58[b]	0.14	0.08	0.91[c]	0.99[c]	—	0.67[c]	−0.02	0.37	0.39
Pheophytin *b*, Pb	0.80[c]	0.76[c]	0.78[c]	0.39	0.12	−0.17	0.79[c]	0.85[c]	0.87[c]	—	−0.09	0.54[a]	0.47[a]
Chl *a*, Ca	0.56[b]	0.52[a]	0.59[b]	0.82[c]	0.49[a]	0.41	0.52[a]	0.40	0.33	0.26	—	0.55[b]	0.10
Chl *b*, Cb	0.93[c]	0.61[b]	0.70[c]	0.70[c]	0.35	0.01	0.67[c]	0.68[c]	0.63[b]	0.70[c]	0.64[b]	—	0.37
Chl *c*, Cc	0.66[c]	0.88[c]	0.85[c]	0.46[a]	0.42	0.16	0.85[c]	0.74[c]	0.76[c]	0.78[c]	0.40	0.58[b]	—

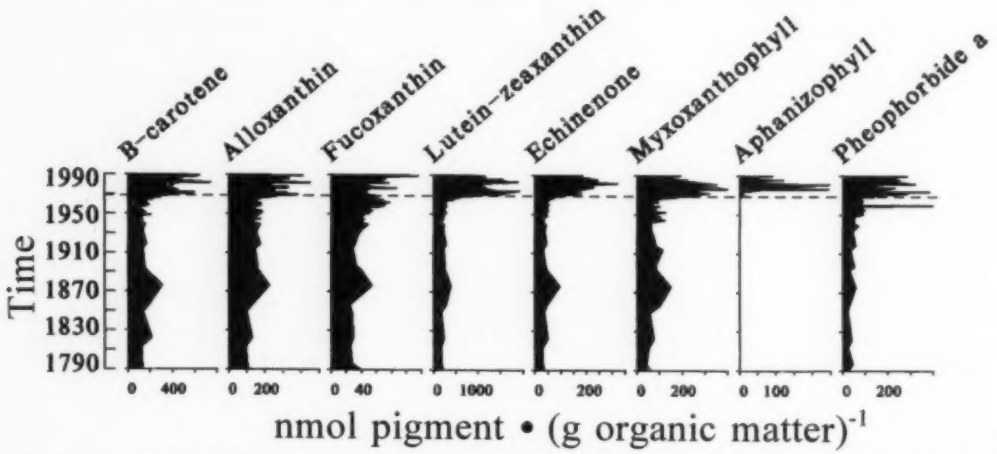

FIG. 4. Concentration profiles for selected fossil pigments in Lake 227 sediments, 1790–1990. All concentrations are nmol pigment · (g organic matter)$^{-1}$. Pigments include β-carotene (all plants), alloxanthin (cryptophytes), fucoxanthin (chrysophytes + diatoms), lutein–zeaxanthin (greens + cyanobacteria), echinenone (total cyanobacteria), myxoxanthophyll (colonial cyanobacteria), aphanizophyll (N_2-fixing cyanobacteria), and total pheophorbide *a* (from herbivory). Broken line = 1969, when nitrogen and phosphorus additions began.

correlated with β-carotene, a stable indicator of total algal biomass.

Pigments from different algal groups were generally uncorrelated (Table 2). For example, fucoxanthin from diatoms and chrysophytes was uncorrelated with pigments from cyanobacteria (myxoxanthophyll, echinenone, aphanizophyll) and chlorophytes (lutein–zeaxanthin, pheophytin *b*, Chl *b*). Lutein–zeaxanthin was only correlated with *b*-phorbins from green algae and stable indicators of total algal biomass. Independence of fossils from different groups is desirable because it indicates that pigments are responding to changes in algal abundance, rather than common environmental conditions that affect preservation (e.g., oxygen, light, temperature, grazers).

Comparison of Fossil Measures

Accumulation rates of pigments were generally poor predictors of algal biomass (Table 1). Only accumulations of stable pigments from green algae (pheophytin *b*, lutein–zeaxanthin) were significantly correlated to standing crop. In addition, accumulation rates were highly correlated among all pigments (Table 2). For example, correlations between fucoxanthin accumulation and accumulation of other pigments were all significant ($r = 0.52–0.96$, $P < 0.05$), except for pigments from filamentous cyanobacteria (aphanizophyll, myxoxanthophyll; Table 2). Accumulations of most pairs of pigments exhibited a mean r of 0.7–0.9 ($P < 0.001$). Such high redundancy reduces the independent information gained from analysis of individual pigments and obscures changes in phytoplankton community composition.

Accumulation rates of several fossil pigments were correlated with planktonic Chl standing crop in Lake 227. Significant correlations were recorded for fucoxanthin ($r = 0.64$, $P < 0.01$) and Chl *c* ($r = 0.74$, $P < 0.001$) from siliceous algae, alloxanthin ($r = 0.55$, $P < 0.05$) and α-carotene ($r = 0.63$, $P < 0.01$) from cryptophytes, pheophytin *b* ($r = 0.66$, $P < 0.01$) from green algae, and ubiquitous β-carotene ($r = 0.65$, $P < 0.01$) and pheophytin a ($r = 0.68$, $P < 0.01$). In addition, Chl standing crop was correlated with bulk organic matter accumulation rates ($r = 0.58$, $P < 0.01$), organic content

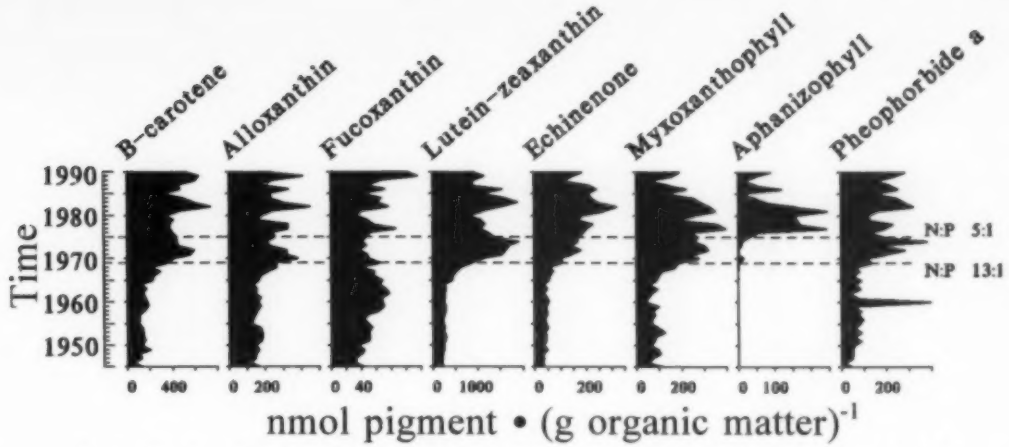

FIG. 5. Concentration profiles for selected fossil pigments in Lake 227 sediments, 1945–1990. Concentrations and pigment codes as in Fig. 4. Lower broken line = 1969, when nitrogen (N) and phosphorus (P) additions at 13:1 mass ratio began; upper broken line = 1975, when N and P were added at a 5:1 mass ratio.

of the sediments ($r = 0.46$, $P < 0.05$), and more weakly to total C fixation ($r = 0.44$, $P = 0.08$; Fig. 2).

Interpretation of the Fossil Record

Premanipulation Era

Baseline concentrations of all sedimentary pigments were stable from 1790 until experimental fertilization began in 1969 (Fig. 4). Twofold increases in concentrations of most pigments near 1875 correspond to the presence of charcoal in varves between 1840 and 1900 (Wolfe et al. 1994) and suggest the influence of fire. Fire scars on local red pine and the ages of jack pine both confirm that the last major regional fire occurred ca 1875 (D. Schindler, Department of Zoology, University of Alberta, Edmonton, Alta. personal communication). Although our temporal resolution was coarse for this point in time (ca. 20 yr), pigment concentrations appear to have quickly declined to predisturbance values by 1900 and did not vary greatly until fertilization in 1969.

Carotenoids and chlorophylls showed little sign of long-term, postdepositional degradation or derivative formation prior to fertilization (Fig. 4). Derivatives of chlorophyllous pigments (pheophytins, pheophorbides) did not become more abundant in older sediments, as would be expected if labile Chl *a* and Chl *c* were converted to more stable pheopigments after burial (Hurley and Armstrong 1991).

As in some Wisconsin lakes (Leavitt et al. 1989, 1993), alloxanthin (cryptophytes) and lutein–zeaxanthin (chlorophytes–cyanobacteria) were generally the most abundant carotenoids in prefertilization sediments. In contrast, dinoflagellates were not represented in the fossil record, probably because of severe degradation of their carotenoid peredinin (Leavitt and Carpenter 1990b; Hurley and Armstrong 1991). Similarly, Chl *c* was rare in prefertilization sediments of Lake 227. Undegraded Chl *c* has been recommended as a paleolimnological indicator of deepwater blooms of chrysophytes (Leavitt and Carpenter 1990a, 1990b); consequently, the very low fossil concentrations of Chl *c* seen here suggest an absence of metalimnetic populations.

Herbivory by zooplankton was relatively constant prior to 1969, as measured by the grazing indicator pheophorbide *a* (Daley 1973; Carpenter and Bergquist 1985). Anomalously high concentrations of pheophorbide *a* in 1960 were confirmed by replicate analyses (coefficient of variation <20%). No other pigments were unusual during 1960 and fossil zooplankton abundance was at a minimum at this time (Hann et al. 1994). Presently, we have no explanation for the unusual pheophorbide concentration.

Fossil Record of Eutrophication

Sedimentary pigments recorded many of the major changes in phytoplankton communities resulting from the 10-fold increase in P loading beginning in 1969 (Fig. 5). By 1973, total algal biomass had increased between two- and fourfold depending on whether Chl *a* (not shown) or β-carotene profiles were interpreted. Concentrations of most taxon-specific carotenoids also increased after fertilization, with the exception of aphanizophyll from N_2-fixing cyanobacteria, and fucoxanthin from diatoms and chrysophytes.

Green algae (as lutein–zeaxanthin, Chl *b*, pheophytin *b*) and cyanobacteria (as echinenone), especially filamentous forms (as myxoxanthophyll), showed the greatest response to eutrophication (Fig. 5). Concentrations of these representative fossil pigments increased 4- to 10-fold during 1970–1976. Concomitant increases in the grazing indicator pheophorbide *a* suggest that herbivory was generally higher through this period, probably because of greens rather than cyanobacteria, which are often inedible when filamentous. Although some changes immediately preceded fertilization, these patterns might have resulted from slight mixing of varves during sediment sampling.

Other members of the premanipulation phytoplankton community may have been less affected by experimental eutrophication than were chlorophytes or cyanobacteria. Populations of cryptophytes (as alloxanthin, α-carotene) only doubled after fertilization. Fucoxanthin from siliceous algae declined in comparison with the early 1960s (Fig. 5), although poor plankton–pigment correlations (Table 1) make it difficult to interpret this change. Similarly, while dinoflagellates were recorded by peredinin (not shown), postfertilization concentrations of this carotenoid were too

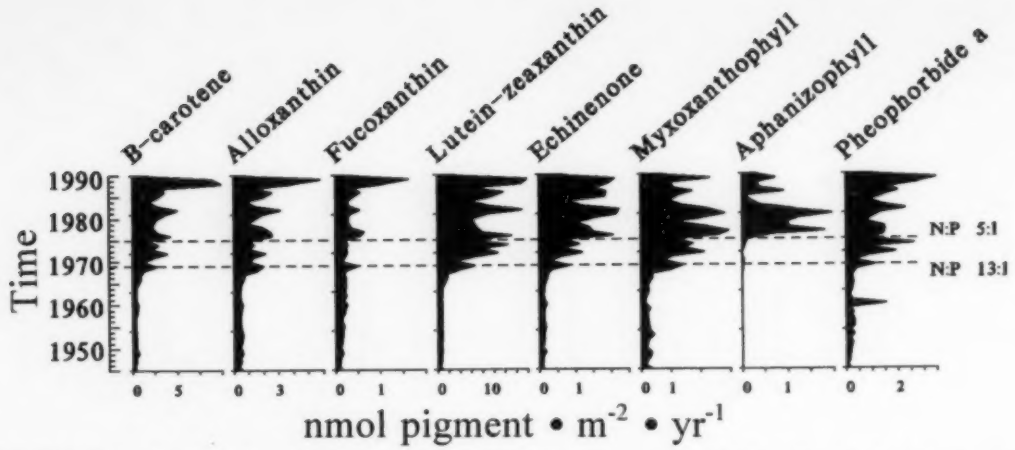

FIG. 6. Accumulation profiles for selected fossil pigments in Lake 227 sediments, 1945–1990. All accumulation rates as nmol pigment · m^{-2} · yr^{-1}. Pigment codes as in Fig. 4 and broken lines as in Fig. 5.

low (<5 nmol pigment · (g organic matter)$^{-1}$) to be reliably distinguished from other minor fossil compounds.

The fossil record recorded changes in the phytoplankton community resulting from reduced N loading in 1975 (Fig. 5). Pigments from green algae were replaced by aphanizophyll from N_2-fixing cyanobacteria. Cyanobacteria remained important in the phytoplankton during the late 1970s, as indicated by consistently high concentrations of fossil echinenone and myxoxanthophyll (Fig. 5).

Algal carotenoids also revealed complex algal community changes during the 1980s. The historical maximum of algal biomass occurred in 1982 and was recorded by elevated concentrations of most carotenoids and Chls (Fig. 5). However, algal populations were unstable, and concentrations of pigments from N_2-fixing cyanobacteria (aphanizophyll) and colonial cyanobacteria (myxoxanthophyll) declined in the late 1980s. This observation, combined with sustained high concentrations of echinenone (derived from all cyanobacteria), suggests that single-cell cyanobacteria increased relative to colonial species in recent years. Post-1982 increases in the grazing indicator pheophorbide *a* also suggest a rise in small edible cells at the expense of filamentous colonies. Unfortunately, this interpretation cannot easily be tested because picoplanktonic cyanobacteria have only been enumerated since 1980 (D.L. Findlay, unpublished data).

Concentrations of pigments from cryptophytes and siliceous algae were highest in the most recent sediments (Fig. 5). Although these results could be interpreted as signalling a partial return of Lake 227 to premanipulation species composition, sedimentary patterns are also similar to those produced by pigment degradation (Leavitt et al. 1993). Incomplete decomposition of pigments during sinking can lead to elevated fossil abundance in the most recently deposited sediments (Leavitt and Carpenter 1990a, 1990b; Hurley and Armstrong 1991) and labile pigments can continue to degrade after deposition. This transient degradation is usually complete within several years, but it reduces the reliability of paleoecological interpretations in very recent deposits (Hurley and Armstrong 1991).

Pigment accumulation rates generally showed the same pattern as stratigraphies based on fossil concentration (Fig. 6). All major taxonomic replacements remained interpretable in accumulation profiles. However, interpretations based on pigment accumulation rates underestimated initial lake response to fertilization in comparison with those based on concentration profiles. Similarly, recent algal abundance (1988–1990) was greater when recorded as pigment accumulation rate than when recorded as fossil concentration. Once again, patterns of high pigment accumulation in surface sediments may result from rapid in situ pigment degradation and are difficult to interpret.

Annual pigment accumulation was more strongly influenced by changes in pigment concentration than by changes in bulk organic matter accumulation. Rates of organic matter accumulation ranged from 5 to 10 mg · cm^{-2} · yr^{-1} between 1790 and 1960, and were similar to those determined by Wolfe et al. (1994). Organic matter accumulation increased twofold immediately after fertilization and remained high but variable throughout most of the manipulation (Fig. 7 in Wolfe et al. 1994). In contrast, pigment concentrations increased 4- to 10-fold. Such small increases in organic matter accumulation after fertilization suggest that much of the prefertilization sediment matrix is derived from pigment-poor allochthonous material, as proposed by Gorham and Sanger (1975).

Discussion

Comparison of Phytoplankton and Fossil Records

Fossil concentrations of stable, ubiquitous pigments (β-carotene, pheophytin *a*) were significantly correlated to total phytoplankton biomass, but were unrelated to ^{14}C fixation (Fig. 2, Table 1). This relation is especially remarkable considering that phytoplankton were only sampled during the ice-free period of the year, yet sediment varves contained fossils deposited over the entire year. Similar significant correlations between total algal biomass and stable fossil pigment concentration have also been noted in surveys of lakes along a gradient of productivity (Gorham et al. 1974; Flanney et al. 1982; Guilizzoni et al. 1983; Brenner and Binford 1988).

In contrast to stable pigments, widely distributed Chl *a* was not correlated to any measure of total algal standing crop, probably because it is extremely labile (Leavitt and

Carpenter 1990b). In situ experiments have shown that chlorophylls are rapidly degraded to both colorless and colored derivatives (Leavitt and Carpenter 1990b; Hurley and Armstrong 1991). Undegraded Chls are best preserved if they originate from deepwater populations (Leavitt et al. 1989; Leavitt and Carpenter 1989, 1990b; Hurley and Watras 1991) or benthic algae (P.R. Leavitt, unpublished data).

Poor correlation between fossil pigment concentration and C fixation was partially expected because ^{14}C fixation does not estimate in situ losses and therefore cannot estimate net algal growth. For example, algal standing crop and total C fixation were unrelated in Lake 227 ($r = 0.14$, $P > 0.5$; Fig. 2). Midday algal growth in Lake 227 is often severely C limited (Schindler and Fee 1973). Consequently, radiocarbon addition during incubations may represent a significant additional C source that is not available to lake populations. However, it is unclear whether fossil pigments and C fixation are unrelated in other lakes. For example, Adams and Prentki (1986) have shown an exceptional correspondence between areal primary production and the gross pigment concentration in surface sediments of a large reservoir ($r = 0.97–1.0$, $N = 6$). Weaker, but significant, correlations were reported by Guilizzoni et al. (1983) for 12 Italian lakes.

Fossil concentrations of highly specific pigments from cyanobacteria (echinenone, aphanizophyll) and chlorophytes (pheophytin *b*, lutein–zeaxanthin) were also correlated to the biomass of their respective algal groups (Table 1, Fig. 3). These algae were dominant during the ice-free season when plankton samples were taken (Fig. 1). In contrast, pigments from taxa that are more common under ice (cryptophytes, chrysophytes) were either weakly correlated (alloxanthin, α-carotene) or were uncorrelated with algal biomass (fucoxanthin, Chl *c*). Interpretation of this pattern suggests that sampling during the ice-free season alone may have been insufficient to accurately estimate abundance of algae that have winter populations.

Food-web interactions may have contributed to poor correlations between cryptophyte biomass and concentration of fossil alloxanthin. Mass budgets from other lakes suggest that the deposition of alloxanthin is more influenced by grazing zooplankton than by algal production (Leavitt and Carpenter 1990a). Herbivores incorporate cryptophytes into rapidly sinking zooplankton feces, which selectively transport alloxanthin to sediments, bypassing pigment degradation by sunlight. Consistent with this hypothesis, alloxanthin was the dominant fossil carotenoid in Lake 227 prior to fertilization (Fig. 4), despite a low standing crop of cryptophytes (Schindler et al. 1973). In addition, fossil alloxanthin concentrations were highly correlated with the abundance of the grazing indicator pheophorbide *a* ($r = 0.76$, $P < 0.001$), a Chl *a* derivative produced exclusively during herbivory (Daley 1973; Carpenter and Bergquist 1985). Unfortunately, year-round plankton samples are required to distinguish between grazing and algal production as controls of cryptophyte deposition in Lake 227.

Correlations between fossil pigment concentration and algal standing crops were similar to those obtained from direct comparisons of pigment and phytoplankton standing crops (Ridout and Morris 1985; Foy 1987; Leavitt and Carpenter 1990a). In both instances, species succession and the presence of pigment-containing detrital material (e.g., moribund cells, resuspended sediments) can independently alter pigment abundance and estimated algal biomass (Leavitt and Carpenter 1990a). For example, chlorophyte biomass declined steadily from 1972 to 1975, whereas fossil pheophytin *b* concentration showed a second peak in 1974 (Fig. 3). Chlorophyte assemblages during that time were characterized by rapid changes in the dominant species (*Scenedesmus denticulatus*, *Ankistrodesmus falcatus* var. *spiralis*, and *Oocystis submarina* var. *variabilis*; Findlay and Kling 1975; Findlay 1978). Algal species differ in their cellular quotas of carotenoids and chlorophylls (Goodwin 1980) and in rates of pigment loss during senescence (Leavitt and Carpenter 1990b). Therefore, changes in species composition can alter the deposition of pigments without affecting total algal biomass, or vice versa.

Errors in sediment sampling may also have reduced correlations between fossil pigments and algal abundance. Subannual laminae in recent sediments may have introduced errors in identification and isolation of individual varves (e.g., Wolfe et al. 1994). In addition, our technique does not completely isolate individual varves and can lead to some mixing between adjacent years. However, the observation that most fossil peaks match those of phytoplankton biomass suggest that sampling error was rarely severe. Occasional mismatches of fossil–biomass peaks (e.g., aphanizophyll – N_2-fixing cyanobacteria in 1981–1982; Fig. 3) may reflect sampling error, or may simply result from continued algal blooms under ice cover, after plankton sampling ceased. Presently, we cannot distinguish between these possibilities.

Preservation of Pigments

Preservation of fossil pigments varies with the deepwater environment and is reduced under conditions of high light, oxygen, temperature, and turbulence (Welschmeyer and Lorenzen 1985a, 1985b; Carpenter et al. 1986, 1988; Leavitt and Carpenter 1990a, 1990b; Hurley and Armstrong 1991). Because a high algal standing crop tends to produce conditions that favor pigment preservation (Swain 1985), it is useful to try and distinguish between production and preservation as factors that control fossil pigment abundance.

Preservation effects clearly dominate fossil abundance of Chl *c* and peredinin in Lake 227. These compounds are only recorded in sediments from the postfertilization era. Experimental studies have demonstrated that both are often completely degraded before deposition (Repeta and Gagosian 1987; Leavitt and Carpenter 1990b; Hurley and Armstrong 1991; Bianchi et al. 1991). Residual pigments that survive deposition are often mineralized in surface sediments within a few years (Repeta and Gagosian 1987; Furlong and Carpenter 1988; Hurley and Armstrong 1991). Consequently, these pigments can be used to confirm the presence of phytoplankton groups, but not to estimate population abundance (e.g., Leavitt et al. 1989, 1993). Similarly, absence of labile Chl *c* or peredinin does not constitute proof that siliceous algae or dinoflagellates were rare.

Among other pigments, two lines of evidence suggest that preservation effects are only occasionally important and that fossil concentrations respond mainly to changes in algal standing crop in Lake 227. First, the 4- to 10-fold increases in fossil pigment concentration at the onset of experimental fertilization (Fig. 5; 1969–1974) were slightly less than the estimated increase in algal biomass (Schindler et al. 1971, 1973). We would expect a larger fossil response if pigment preservation had increased greatly after fertilization. Preservation conditions may not have changed much with eutrophication

because the hypolimnion of Lake 227 was naturally anoxic prior to fertilization (Schindler et al. 1971, 1973).

Second, fossil preservation increased during the algal biomass maximum of 1982, although not during other large blooms. Mineralization of organic matter from blooms can reduce deepwater oxygen levels and increase preservation of all pigments independent of the production of individual algal groups. For example, in 1982 only filamentous cyanobacteria bloomed (65 g wet wt · m^{-2}), yet pigments from all algal groups showed pronounced peaks in fossil concentration (Figs. 3 and 5). In contrast, chlorophyte blooms reached 45 g · m^{-2} (wet wt) during 1972–1973, yet only pigments from green algae showed high fossil concentrations. It is unclear why cyanobacteria alone should be particularly good at promoting preservation of all fossil pigments, but our finding suggests that preservation artifacts may be less important during noncyanobacterial blooms.

Pigment Accumulation and Concentration

Fossil pigment concentration (nmol pigment · (g organic matter)$^{-1}$) was a better measure of phytoplankton abundance than was pigment accumulation rate (nmol pigment · m^{-2} · yr^{-1}) for interpretation of algal community change in Lake 227. Accumulation rates of pigments were uncorrelated with both algal biomass and C fixation. Furthermore, accumulation rates of pigments from unrelated algal groups were highly redundant (Table 2). Discrepancies between accumulation rates and fossil concentrations may have arisen either because of difficulty in estimating annual bulk sediment accumulation rates, or because of postdepositional decay of pigments in surface sediments.

Pigment accumulation rates were calculated as the product of pigment concentration, organic content, and bulk sediment accumulation. Replicate determinations of organic content agreed within 10%. Consequently, pigment concentration and accumulation rate differed only by bulk sediment flux. Accurate quantification of annual bulk accumulation requires complete isolation of a single varve below a known sediment surface area, a difficult task. Thus, although estimates obtained here generally agreed with those from independent, photographic analysis of cores in 1988 (Wolfe et al. 1994), sampling error may have reduced the accuracy of fossil pigment accumulation rate determinations. Errors in estimation of bulk sediment flux could also contribute to spurious correlations between unrelated pigments because individual accumulation rates are all calculated as the product of pigment concentration and total sediment accumulation.

Degradation of carotenoids and chlorophylls in sediments deposited after 1987 also may have biased pigment accumulation rates more so than fossil concentrations. Pigment loss often continues after deposition (Furlong and Carpenter 1988; Repeta and Gagosian 1987; Hurley and Armstrong 1991), but can be similar to the rate of total organic C mineralization (Gorham et al. 1974; Swain 1985; Leavitt 1988; Hurley and Armstrong 1991). Consequently, the specific pigment content of organic sediments may be relatively stable through time. In contrast, pigment accumulation rates are estimated independently of organic matter content and should be more affected by continuing in situ pigment decomposition. In a short time series such as ours, this bias could obscure correlations between algal abundance and the pigment accumulation rate. Accumulation rates and fossil concentrations should be equally accurate for pigments or hard fossils that do not degrade relative to bulk organic matter after deposition (see below).

Although fossil pigment concentrations compared well with the historical record of phytoplankton in Lake 227, fossil accumulation rates may be a better measure of algal abundance for comparisons among widely differing lake types. For example, allochthonous inputs of organic matter can vary greatly between watersheds and may lead to inaccurate estimates of algal biomass when fossil concentrations are calculated (P.R. Leavitt, unpublished data). Therefore, if simple interlake comparisons of total algal biomass are desired, pigment accumulation rates may be the best measure, particularly if corrected for postdepositional degradation or focusing (e.g., Hurley et al. 1992). On the other hand, if paleoecological reconstructions of algal community change are needed, investigators should use fossil pigment concentration.

Interpretation of the Fossil Pigment Record

What Do Fossil Pigments Tell Us?

Fossil pigments recorded many of the major responses of phytoplankton to chemical changes in Lake 227 resulting from experimental eutrophication (Fig. 5). Increased nutrient flux was shown by pigments from colonial green algae and cyanobacteria, both of which are characteristic of productive lakes. Similarly, N deficits resulting from threefold declines in N inputs after 1975 were recorded by increases in carotenoids from N_2-fixing cyanobacteria. Pigments summarized phytoplankton community change in units equivalent to functional groups of algae (edible, colonies, N_2-fixing, deep blooms, siliceous). These are the units most often used by ecologists when dealing with long time series (e.g., Schindler et al. 1985; Kitchell 1992; Carpenter and Kitchell 1993). In addition, fossil pigments provided information that could not be obtained by other means.

Fossil pigments confirmed the significance of fertilization and provided a framework for evaluating environmental change and disturbance over a long time period. Fossil concentrations changed little between 1790 and 1967 and were intermediate to the high values characteristic of eutrophic or meromictic lakes (Brown et al. 1984; Züllig 1981, 1989; Leavitt et al. 1993) and the low levels common in unproductive alpine and arctic sites (P.R. Leavitt and D. Schindler, unpublished data) (Fig. 4). In fact, photographic analysis of varve thickness suggests that lake conditions were constant as far back as 1650 AD (Wolfe et al. 1994). Paleoecological analysis of pigments suggested that eutrophication was the most significant event in the last 350 yr of Lake 227.

Fossil pigments also identified historical fertilization events, such as those caused by forest fires, that could be used for comparison with experimental fertilization. Peak pigment concentrations near 1875 corresponded to sedimentary charcoal and were substantially less than those during eutrophication (post-1969). In addition, paleolimnological analysis revealed that fire effects were short-lived (Fig. 4), which is consistent with results from fires near Lake 226 (Schindler et al. 1980), Lake 239 (Bayley et al. 1992), and experimental deforestation in Hubbard Brook (Likens et al. 1970). Together, these studies suggest that experimental fertilization was a more significant perturbation than forest fire and was a very powerful test of the P-limitation hypothesis.

What Do Fossil Pigments Not Tell Us?

Fossil carotenoids and Chls could not be used to accurately estimate the abundance of certain algal groups. Peridinin from dinoflagellates and Chl *c* from siliceous algae were poorly preserved under all conditions, which is consistent with experimental demonstration of their lability (Leavitt and Carpenter 1990b; Hurley and Armstrong 1991). Because of such selective deposition, investigators should avoid interpreting changes in the relative abundance of fossil pigments (e.g., percentage of total pigments). Instead, fossil pigment interpretations should be based on historical changes in pigment concentration (e.g., green algae change relative to the history of green algae).

Although our techniques achieved annual resolution, comparison of individual years within the stratigraphic record was difficult unless there was a significant historical event (e.g., 1982). Weak fossil signals (peak/baseline <1.5; Leavitt and Carpenter 1989) may be uninterpretable because of substantial unexplained variation in plankton–fossil correlations. In addition, incomplete isolation of adjacent varves and the presence of subannual laminae in sediments of the fertilization era (Wolfe et al. 1994) may have caused some mixing of adjacent years (e.g., 1968 and 1969; Fig. 5). While other sampling methods (tape peel, seasonal sectioning) may provide superior time resolution (Simola 1977; Davidson 1988), these techniques are difficult to apply quantitatively for pigments.

Interpretation of recent historical events (<3–5 yr old) was difficult using fossil pigments. Elevated abundance of several pigments during 1988–1989 was consistent with high Chl standing crops (Fig. 2), but could not be reliably distinguished from incomplete in situ degradation (Figs. 5 and 6). Pigment degradation is normally most intense during sinking through the water column (Welshmeyer and Lorenzen 1985b; Carpenter et al. 1986, 1988; Leavitt et al. 1990a, 1990b). However, losses in water and sediments are partially compensatory, and residual pigments can continue to decompose in surface deposits (Furlong and Carpenter 1988; Hurley and Armstrong 1991). The magnitude of degradation will depend on the degree of sediment mixing, zoobenthos activity, oxygen content of sediments, and bulk accumulation rate. However, once below the zone of active loss, fossil preservation can be excellent for hundreds of years (Fig. 4). If interpretations of surface deposits must be conducted, investigators should use organic-specific fossil concentration rather than accumulation rates, because mineralization of labile bulk organics partly compensates for pigment degradation (Swain 1985) and reduces the magnitude of bias in surface sediments (Fig. 5 vs. Fig. 6).

In conclusion, several fossil pigments were reliable indicators of algal response to lake eutrophication. Ubiquitous stable pigments were particularly accurate for reconstruction of total algal biomass, while taxon-specific carotenoids recorded changes in the main algal functional groups (edible, colonial, algal division, N_2-fixing, etc.). Although fossil pigments primarily recorded changes in algal biomass, fossil concentrations were also influenced by species replacement within functional groups and by fossil degradation. Unfortunately, the utility of pigments from siliceous algae and cryptophytes is still unresolved. In addition, further studies are needed to evaluate pigment reliability under different environmental conditions (acidification, salinization) and in deep, oxygenated, transparent lakes where degradation processes are more important.

Acknowledgments

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References

Adams, M.S., and R.T. Prentki. 1986. Sedimentary pigments as an index of the trophic status of Lake Mead. Hydrobiologia 143: 71–77.

Bayley, S.E., D.W. Schindler, K.G. Beaty, B.R. Parker, and M.P. Stainton. 1992. Effects of multiple fires on nutrient yields from streams draining boreal forest and fen watersheds: nitrogen and phosphorus. Can. J. Fish. Aquat. Sci. 49: 584–596.

Bianchi, T.S., S. Findlay, and D. Fontvieielle. 1991. Experimental degradation of plant materials in Hudson River sediments. I. Heterotrophic transformations of plant pigments. Biogeochemistry 12: 171–187.

Beamish, R.J., L.M. Blouw, and G.A. McFarlane. 1976. A fish and chemical study of 109 lakes in the Experimental Lakes Area, northwestern Ontario, with appended reports on lake whitefish ageing errors and northwestern Ontario baitfish industry. Can. Fish. Mar. Serv. Tech. Rep. 607: 116 p.

Brenner, M., and M.W. Binford. 1988. Relationships between concentrations of sedimentary variables and trophic state in Florida lakes. Can. J. Fish. Aquat. Sci. 45: 294–300.

Brown, S.R. 1969. Paleolimnological evidence from fossil pigments. Mitt. Int. Ver. Theor. Angew. Limnol. 17: 95–103.

Brown, S.R., H.J. McIntosh, and J.P. Smol. 1984. Recent paleolimnology of a meromictic lake: fossil pigments of photosynthetic bacteria. Verh. Int. Ver. Theor. Angew. Limnol. 22: 1357–1360.

Brunskill, G.J., and D.W. Schindler. 1973. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139–155.

Carpenter, S.R., and A.M. Bergquist. 1985. Experimental tests of grazing indicators based on chlorophyll *a* degradation products. Arch. Hydrobiol. 102: 303–317.

Carpenter, S.R., M.M. Elser, and J.J. Elser. 1986. Chlorophyll production, sedimentation and degradation: implications for paleolimnology. Limnol. Oceanogr: 31: 112–124.

Carpenter, S.R., and J.F. Kitchell [ed.]. 1993. The trophic cascade in lakes. Cambridge University Press, Cambridge, Mass. 385 p.

Carpenter, S.R., P.R. Leavitt, J.J. Elser, and M.M. Elser. 1988. Chlorophyll budgets: response to food web manipulations. Biogeochemistry 6: 79–90.

Daley, R.J. 1973. Experimental characterization of lacustrine chlorophyll diagenesis. II. Bacterial, viral and herbivore grazing effects. Arch. Hydrobiol. 72: 409–439.

Davidson, G.A. 1988. A modified tape-peel technique for preparing permanent qualitative microfossil slides. J. Paleolimnol. 1: 229–234.

Dean, W.E. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. J. Sediment. Petrol. 44: 242–248.

Fee, E.J. 1973. Modelling primary production in water bodies: a numerical approach that allows vertical inhomogeneities. J. Fish. Res. Board Can. 30: 1469–1473.

Fee, E.J. 1980. Important factors for estimating annual phytoplankton production in the experimental Lakes Area. Can. J. Fish. Aquat. Sci. 37: 513–522.

Findlay, D.L. 1978. Species successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1974–1976. Can. Fish. Mar. Serv. MS Rep. 1466: 41 p.

Findlay, D.L. 1981. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Data from 1977 to 1979. Can. MS Rep. Fish. Aquat. Sci. 1627: 40 p.

Findlay, D.L., R.E. Hecky, L.L. Hendzel, M.P. Stainton, and G.W. Regehr. 1994. Relationship between N_2-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. Can. J. Fish. Aquat. Sci. 51: 2254–2266.

FINDLAY, D.L., AND S.E.M. KASIAN. 1987. Phytoplankton community responses to nutrient addition in Lake 226, Experimental Lakes Area, northwestern Ontario. Water Air Soil Pollut. 30: 719–726.

FINDLAY, D.L., AND H.J. KLING. 1975. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario, following artificial eutrophication. Can. Fish. Mar. Serv. Tech. Rep. 513: 53 p.

FLANNEY, M.S., R.D. SNODGRASS, AND T.J. WHITMORE. 1982. Deepwater sediments and trophic conditions in Florida lakes. Hydrobiologia 92: 597–602.

FOY, R.H. 1987. A comparison of chlorophyll *a* and carotenoid concentrations as indicators of algal volume. Freshwater Biol. 17: 237–250.

FURLONG, E.T., AND R. CARPENTER. 1988. Pigment preservation and remineralization in oxic coastal marine sediments. Geochim. Cosmochim. Acta 52: 87–99.

GOODWIN, T.W. 1980. The biochemistry of the carotenoids. Vol. 1. Plants. Chapman and Hall, New York, N.Y.

GORHAM, E., J.W.G. LUND, J.E. SANGER, AND W.E. DEAN, JR. 1974. Some relationships between algal standing crop, water chemistry, and sediment chemistry in the English Lakes. Limnol. Oceanogr. 19: 601–617.

GORHAM, E., AND J. SANGER. 1975. Fossil pigments in Minnesota lake sediments and their bearing upon the balance between terrestrial and aquatic inputs to sedimentary organic matter. Verh. Int. Ver. Theor. Angew. Limnol. 19: 2267–2273.

GUILIZZONI, P., G. BONOMI, G. GALANTI, AND D. RUGGIU. 1983. Relationship between sedimentary pigments and primary production: evidence from core analyses of twelve Italian lakes. Hydrobiologia 103: 103–106.

HANN, B.J., P.R. LEAVITT, AND P.S.S. CHANG. 1994. Cladocera community response to experimental eutrophication in Lake 227 as recorded in laminated sediments. Can J. Fish. Aquat. Sci. 51: 2312–2321.

HURLEY, J.P., AND D.E. ARMSTRONG. 1991. Pigment preservation in lake sediments: a comparison of sedimentary environments in Trout Lake, Wisconsin. Can. J. Fish. Aquat. Sci. 48: 472–486.

HURLEY, J.P., D.E. ARMSTRONG, AND A.L. DUVALL. 1992. Historical interpretation of pigment stratigraphy in Lake Mendota sediments, p. 49–68. *In* S.R. CARPENTER AND J.F. KITCHELL [ed.] The trophic cascade in lakes. Cambridge University Press, Cambridge, Mass.

HURLEY, J.P., AND C.J. WATRAS. 1991. Identification of bacteriochlorophylls in lakes via reverse-phase HPLC. Limnol. Oceanogr. 36: 307–315.

KITCHELL, J.F. [ed.]. 1992. Food web management: a case study of Lake Mendota. Springer-Verlag, New York, N.Y. 553 p.

KLING, H.J., D.L. FINDLAY, AND J. KOMAREK. 1994. *Aphanizomenon schindleri* sp.nov.: a new nostocacean cyanoprokaryote from the Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 51: 2267–2273.

KLING, H.J., AND S.K. HOLMGREN. 1972. Species composition and seasonal distribution of phytoplankton in the Experimental Lakes Area, northwestern Ontario. Can. Fish. Mar. Serv. Tech. Rep. 337: 5 p.

LEAVITT, P.R. 1988. Experimental determination of carotenoid degradation. J. Paleolimnol. 1: 215–228.

LEAVITT, P.R., AND S.R. CARPENTER. 1989. Effects of sediment mixing and benthic algal production on fossil pigment stratigraphies. J. Paleolimnol. 2: 147–158.

LEAVITT, P.R., AND S.R. CARPENTER. 1990a. Regulation of pigment sedimentation by photo-oxidation and herbivore grazing. Can. J. Fish. Aquat. Sci. 47: 1166–1176.

LEAVITT, P.R., AND S.R. CARPENTER. 1990b. Aphotic pigment degradation in the hypolimnion: implications for sedimentation studies and paleolimnology. Limnol. Oceanogr. 35: 520–535.

LEAVITT, P.R., S.R. CARPENTER, AND J.F. KITCHELL. 1989. Whole-lake experiments: the annual record of fossil pigments and zooplankton. Limnol. Oceanogr. 34: 700–717.

LEAVITT, P.R., P.R. SANFORD, S.R. CARPENTER, J.F. KITCHELL, AND D. BENKOWSKI. 1993. Annual fossil records of food-web manipulation, p. 278–309. *In* S.R. CARPENTER AND J.F. KITCHELL [ed.] The trophic cascade in lakes. Cambridge University Press, Cambridge, Mass.

LIAAEN-JENSEN, S. 1971. Isolation, reactions, p. 61–188. *In* O. ISLER [ed.] Carotenoids. Birkhausser Verlag, New York, N.Y.

LIKENS, G.E., F.H. BORMANN, N.M. JOHNSON, D.W. FISHER, AND R.S. PIERCE. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in Hubbard Brook watershed-ecosystem. Ecol. Monogr. 40: 23–47.

MALLEY, D.F., P.S.S. CHANG, D.L. FINDLAY, AND D.A. LINSEY. 1988. Extreme perturbation of the zooplankton community of a small Precambrian Shield lake by the addition of nutrients. Verh. Int. Ver. Theor. Angew. Limnol. 23: 2237–2247.

MANTOURA, R.F.C., AND C.A. LLEWELLYN. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse phase high-performance liquid chromatography. Anal. Chim. Acta 151: 297–314.

NAUWERCK, A. 1963. Die Beziehungen zwischen Zooplankton und Phytoplankton in see Erken. Symb. Bot. Ups. 17: 163 p.

PATALAS, K. 1971. Crustacean plankton communities in forty-five lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 231–244.

REPETA, D.J., AND R.B. GAGOSIAN. 1987. Carotenoid diagenesis in recent marine sediments. I. The Peru continental shelf (15°S, 75°W). Geochim. Cosmochim. Acta 51: 1001–1009.

RIDOUT,P.S., AND R.J. MORRIS. 1985. Short-term variations in the pigment composition of a spring phytoplankton bloom from an enclosed experiment. Mar. Biol. 87: 7–11.

SANGER, J.E. 1988. Fossil pigments in paleoecology and paleolimnology. Palaeogeogr. Palaeoclimatol. Palaeoecol. 62: 343–359.

SCHINDLER, D.W. 1971. Light, temperature and oxygen regimes of selected lakes in the Experimental Lakes Area (ELA), northwestern Ontario. J. Fish. Res. Board Can. 28: 295–301.

SCHINDLER, D.W. 1977. Evolution of phosphorus limitation in lakes: natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. Science (Wash., D.C.) 195: 260–262.

SCHINDLER, D.W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 6–25.

SCHINDLER, D.W., F.A.J. ARMSTRONG, S.K. HOLMGREN, AND G.J. BRUNSKILL. 1971. Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by the addition of phosphate and nitrate. J. Fish. Res. Board Can. 28: 1763–1782.

SCHINDLER, D.W., AND E.J. FEE. 1973. Diel variation in dissolved inorganic carbon and its use in estimating primary production and CO_2 invasion in Lake 227. J. Fish. Res. Board Can. 30: 1501–1511.

SCHINDLER, D.W., H. KLING, R.V. SCHMIDT, J. PROKOPOWICH, V.E. FROST, R.A. REID, AND M. CAPEL. 1973. Eutrophication of Lake 227 by addition of phosphorus and nitrate. The second, third, and fourth years of enrichment, 1970, 1971, and 1972. J. Fish. Res. Board Can. 30: 1415–1440.

SCHINDLER, D.W., K.W. MILLS, D.F. MALLEY, D.L. FINDLAY, J.A. SHEARER, I.J. DAVIES, M.A. TURNER, G.A. LINSEY, AND D.R. CRUIKSHANK. 1985. Long-term ecosystem stress: the effects of years of acidification of a small lake. Science (Wash., D.C.) 228: 1395–1401.

SCHINDLER, D.W., R.W. NEWBURY, K.G. BEATY, J. PROKOPOWICH, T. RUSZCZYNSKI, AND J.A. DALTON. 1980. Effects of a windstorm and forest fire on chemical losses from forested watersheds and on the quality of receiving streams. Can. J. Fish. Aquat. Sci. 37: 328–334.

SHEARER, J.A. 1978. Two devices for obtaining water samples integrated over depth. Can. Fish. Mar. Serv. Tech. Rep. 772: 9 p.

SHEARER, J.A., E.R. DEBRUYN, D.R. DECLERCQ, D.W. SCHINDLER, AND E.J. FEE. 1985. Manual of phytoplankton primary production methodology. Can. Tech. Rep. Fish. Aquat. Sci. 1341: 58 p.

SHEARER, J.A., E.J. FEE, E.R. DEBRUYN, AND D.R. DECLERCQ. 1987. Phytoplankton productivity changes in a small, double-basin lake in response to the experimental acidification of a small Canadian Shield Lake. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 47–54.

SIMOLA, H. 1977. Diatom succession in the formation of annually laminated sediments in Lake Lovojarvi, a small eutrophicated lake. Ann. Bot. Fenn. 14: 35–45.

STAINTON, M.P., M.L. CAPEL, AND F.A.J. ARMSTRONG. 1977. The chemical analysis of fresh water. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

STOCKNER, J.G. 1971. Preliminary characterization of lakes in the Experimental Lakes Area, northwestern Ontario, using diatom occurrences in sediments. J. Fish. Res. Board Can. 28: 265–275.

STOCKNER, J.G., AND F.A.J. ARMSTRONG. 1971. Periphyton of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 215–229.

SWAIN, A.M. 1973. A history of fire and vegetation in northeastern Minnesota as recorded in lake sediments. Quat. Res. 3: 383–396.

SWAIN, E.B. 1985. Measurement and interpretation of sedimentary pigments. Freshwater Biol. 15: 53–75.

VOLLENWEIDER, R.A. 1968. The scientific basis of lake and stream eutrophication, with particular reference to phosphorus and nitrogen as eutrophication factors. O.E.C.D. Paris Tech. Rep. DAS/CSI/68. 27: 182 p.

WELSCHMEYER, N.A., AND C.J. LORENZEN. 1985a. Chlorophyll budgets: zooplankton grazing and phytoplankton growth in a temperate fjord and the Central Pacific Gyres. Limnol. Oceanogr. 30: 1–21.

WELSCHMEYER, N.A., AND C.J. LORENZEN. 1985b. Role of herbivory in controlling phytoplankton abundance: annual pigment budget for a temperate marine fjord. Mar. Biol. 90: 75–86.

WOLFE, B., H.J. KLING, G.J. BRUNSKILL, AND P. WILKINSON. 1994. Multiple dating of a freeze core from Lake 227, an experimentally fertilized lake with varved sediments. Can J. Fish. Aquat. Sci. 51: 2274–2285.

ZEEB, B.A., C.E. CHRISTIE, J.P. SMOL, D.L. FINDLAY, H.J. KLING, AND H.J.B. BIRKS. 1994. Responses of diatom and chrysophyte assemblages in Lake 227 sediments to experimental eutrophication. Can. J. Fish. Aquat. Sci. 51: 2300–2311.

ZÜLLIG, H. 1981. On the use of carotenoids stratigraphy in lake sediments for detecting past developments of phytoplankton. Limnol. Oceanogr. 26: 970–976.

ZÜLLIG, H. 1989. Role of carotenoids in lake sediments for reconstructing trophic history during the late Quaternary. J. Paleolimnol. 2: 23–40.

Responses of Diatom and Chrysophyte Assemblages in Lake 227 Sediments to Experimental Eutrophication[1]

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We examined the diatom and chrysophyte assemblages preserved in Lake 227 sediments prior to, during, and following the annual additions of phosphorus (P) and nitrogen (N) (from 1969–1989). Premanipulation (1957–1968) diatom and chrysophyte assemblages were dominated by taxa characteristic of acidic, unproductive lake conditions. In 1969, the assemblages immediately shifted in response to increased nutrient availability and/or increased pH values in the epilimnion. Large increases in the relative abundance of *Synedra* PIRLA 2 and *S. rumpens* var. *familiaris*, *Mallomonas crassisquama*, *M. doignonii* var. *tenuicostis*, and cysts 1, 15, 29, 233, and 120 largely replaced the prefertilization assemblages. From 1970–1990, the diatom assemblage was dominated by alkaliphilic or pH-indifferent taxa. A second chrysophyte assemblage shift, which coincided with a lowering of the N:P addition ratio in 1975, was characterized by decreases in *M. crassisquama* and cysts 1, 15, 29, 233, and 120, and corresponding increases in *Synura sphagnicola* Korshikov scales and cysts 62 and 33. The stratigraphical analyses and numerical rate-of-change analyses clearly showed that diatom and chrysophyte species composition, particularly chrysophyte cysts, closely tracked short-term changes in lakewater chemistry. The amount of variance in the stratigraphic assemblages explained by lake-water chemical changes was high (68–90%), indicating the close algae–chemistry relationships in Lake 227 from 1969–1989.

Nous avons examiné des restes de diatomées et de chrysophytes contenus dans les sédiments du lac 227 avant, pendant et après l'addition annuelle de phosphore (P) et d'azote (N) (de 1969 à 1989). Avant la transformation du lac (soit de 1957 à 1968), les assemblages étaient dominés par des taxons caractéristiques de conditions lacustres acides et non productives. En 1969, ils ont immédiatement répondu à la plus grande disponibilité d'éléments nutritifs et (ou) à la hausse du pH en zone épiliminique : l'abondance relative de *Synedra* PIRLA 2, de *S. rumpens* var. *familiaris*, de *Mallomonas crassisquama*, de *M. doignonii* var. *tenuicostis* a été accrue, et les spores 1, 15, 29, 233 et 120 ont largement remplacé les groupes qui étaient observés avant le commencement du traitement. De 1970 à 1990, les diatomées étaient dominées par des taxons alcalinophiles ou qui sont indifférents au pH. Un second rééquilibrage des assemblages de chrysophytes, qui a coïncidé avec l'abaissement du taux N/P de la charge en 1975, a été caractérisé par une diminution de *M. crassisquama* et des spores 1, 15, 29, 233 et 120, ainsi que par une hausse correspondante de *Synura sphagnicola*, du groupe de Korshikov et des spores 62 et 33. Les analyses stratigraphiques ainsi que celles du taux de changement ont clairement montré que la composition spécifique en diatomées et en chrysophytes, notamment les spores de chrysophytes, suivaient de près les changements à courte échéance de la chimie de l'eau, qui expliquaient 68 à 90 % de la variance des assemblages stratigraphiques observés dans le lac 227 entre 1969 et 1989.

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Few North American lakes provide the opportunity to compare long-term historical records of water chemistry and phytoplankton populations with paleolimnological records. Moreover, the important question of how closely sedimentary diatom and chrysophyte assemblages track lakewater changes, or whether there are significant lags in response, has rarely been addressed.

Located in the Experimental Lakes Area (ELA), northwestern Ontario, Lake 227 is an ideal location for such a

[1]This paper is one of a series done in honor of the 25th anniversary of the Experimental Lakes Area.

study because it was the site of a whole-lake addition of phosphorus (P) and nitrogen (N) that has been monitored for 24 yr. Numerous studies have been completed on this lake documenting the chemical (Flett et al. 1980; Levine et al. 1986; Schindler et al. 1987) and biological changes in the primary producers (Schindler and Holmgren 1971; Schindler et al 1971; Schindler and Fee 1974; Schindler 1977; Findlay et al. 1994) and zooplankton populations (Patalas 1971; Malley et al. 1988). Furthermore, Lake 227 is an excellent site for a high-resolution paleolimnological investigation because the water column is strongly stratified; the lake has an anoxic hypolimnion, and seldom mixes below 9 m (Schindler et al. 1987). This combination of factors has resulted in annually laminated sediments over the last 400 yr (Wolfe et al. 1994), which provide chronological control.

The purpose of this investigation was to determine how siliceous algal (diatoms and chrysophytes) assemblages preserved in a lake sediment core (representing the period ca. 1957–1990) tracked limnological changes resulting from the fertilization of a whole-lake system, and to compare these assemblages with the long-term phytoplankton history and water chemistry that have been recorded for Lake 227 since 1969.

Materials and Methods

A short freeze core was retrieved from the deepest portion of Lake 227 in January 1990 (Leavitt and Findlay 1994). The outermost 5 mm of sediment was removed from the core and the remaining sediment was freeze dried for 24 h (Leavitt et al. 1989). Laminae corresponding to deposition in one year were separated manually (1945–1990) at −8°C within 3 h of freeze-drying the sediments (Leavitt and Findlay 1994). The sediments were subsampled for the analysis of various chemical and biological indicators.

Sediment samples were prepared according to standard techniques (Smol 1983). Briefly, the freeze-dried sediment was digested in polyethylene centrifuge tubes with a mixture of potassium dichromate and sulphuric acid (1 g $K_2Cr_2O_7$ in 20 mL H_2SO_4). After several days, the material was boiled to complete the digestion and then rinsed eight times with distilled water. Light microscope (LM) slides were prepared by resuspending the cleaned siliceous material and evaporating it onto glass coverslips at 25°C. The dried coverslips were then permanently mounted in Hyrax® on glass slides. The same slides were counted for each of the three microfossil groups investigated (diatoms, chrysophyte scales, and chrysophyte cysts).

Scanning electron microscopy (SEM) was used to develop a working cyst taxonomy for Lake 227. The same sediment slurries used for LM were evaporated onto a smooth piece of aluminum foil, which was affixed to aluminum SEM stubs. Each sample was then sputter-coated with gold and examined at 20kV (working distance = 15 mm).

Diatom and chrysophyte remains were enumerated at 1000× with bright-field and Nomarski optics, respectively. In all cases, micrographs were taken. At least 500 diatom valves and 500 chrysophyte scales were counted along parallel transèct̀s; a minimum of 300 chrysophyte cysts were similarly counted.

Diatom taxonomy generally followed Hustedt (1928–1966, 1930), Cleve-Euler (1951–1955), Patrick and Reimer (1966, 1975), Koppen (1975), Nicholls and Carney (1979), Camburn et al. (1984–1986), and Krammer and Lange-Bertalot (1986). The uncited ecological data discussed for some of the diatom taxa are based on the PIRLA database maintained at the Paleoecological Environmental Assessment and Research Laboratory (e.g., Charles et al. 1990; Kingston et al. 1990).

Relatively few cyst morphotypes have been linked to the taxa that produce them (Smol 1995). In 1986, the International Statospore Working Group (ISWG) published guidelines for cyst descriptions (Cronberg and Sandgren 1986); this has done much to standardize taxonomic research on cysts (e.g., Rybak 1987; Rybak et al. 1987, 1991; Duff and Smol 1988, 1989, 1991; Zeeb et al. 1990; Duff et al. 1992; Carney et al. 1992). Our descriptions of the Lake 227 flora followed the ISWG guidelines and the authorities listed above. Unfortunately, the taxonomic affinities of the dominant cysts recorded in this study have not been determined, but information is available on their ecological optima, based on paleolimnological studies using ISWG guidelines (Duff and Smol 1988, 1989, 1991).

Chrysophyte scale taxonomy followed Asmund and Kristiansen (1986) and Siver (1991b). The ecological data cited came from phytoplankton collections by Siver (1991b), who calculated the abundance-weighted means (AWM) for pH (i.e., the average of all pH values of lakes in which the taxon occurs, weighted by the taxon's relative abundance in each lake) for chrysophyte taxa referred to in the present study. Chemical and phytoplankton data for Lake 227 came from Schindler et al. (1980) and Leavitt et al. (1994).

The microfossil data obtained from the diatom and chrysophyte (cysts and scales) analyses are presented as relative abundance diagrams. Quantitative (i.e., concentration and accumulation rate) analyses were not done because of lack of material from the master core. We used correction factors to proportionally adjust scale counts to cell counts for individual chrysophyte species (see Siver 1991a).

The computer program RATEPOL (J.M. Line, University of Cambridge, Cambridge, U.K.; and H.J.B. Birks, unpublished data) was used to calculate the biostratigraphic rate of change (*sensu* Grimm and Jacobson 1992) in the three different algal data sets. Chord distance (Prentice 1980) between adjacent samples divided by the time interval between the samples provides a simple and robust estimate of the biostratigraphic rate of change per year (Bennett et al. 1992). Estimated rates of change permit comparison of the relative rates of response of the different algal groups to experimental manipulation.

It was useful to compare how total variance in the three algal biostratigraphies for 1969–1989 was explained by the known water chemical changes since 1969. Thus, a series of partially constrained ordination models (Borcard et al. 1992) were designed to partition the variance in the three data sets into four statistically independent fractions: (1) the fraction explained by known water chemical changes independent of any other temporal changes; (2) the fraction explained by water chemical changes that are themselves covarying with time and hence are time-structured; (3) the fraction explained by time independent of any water chemical changes; and (4) the fraction unexplained by water chemistry or by time. Borcard et al. (1992), Legendre (1993), and Lotter and Birks (1993) provide further details of this variance-partioning procedure. Computations were done using the computer program CANOCO version 3.12 (ter Braak 1987–1990).

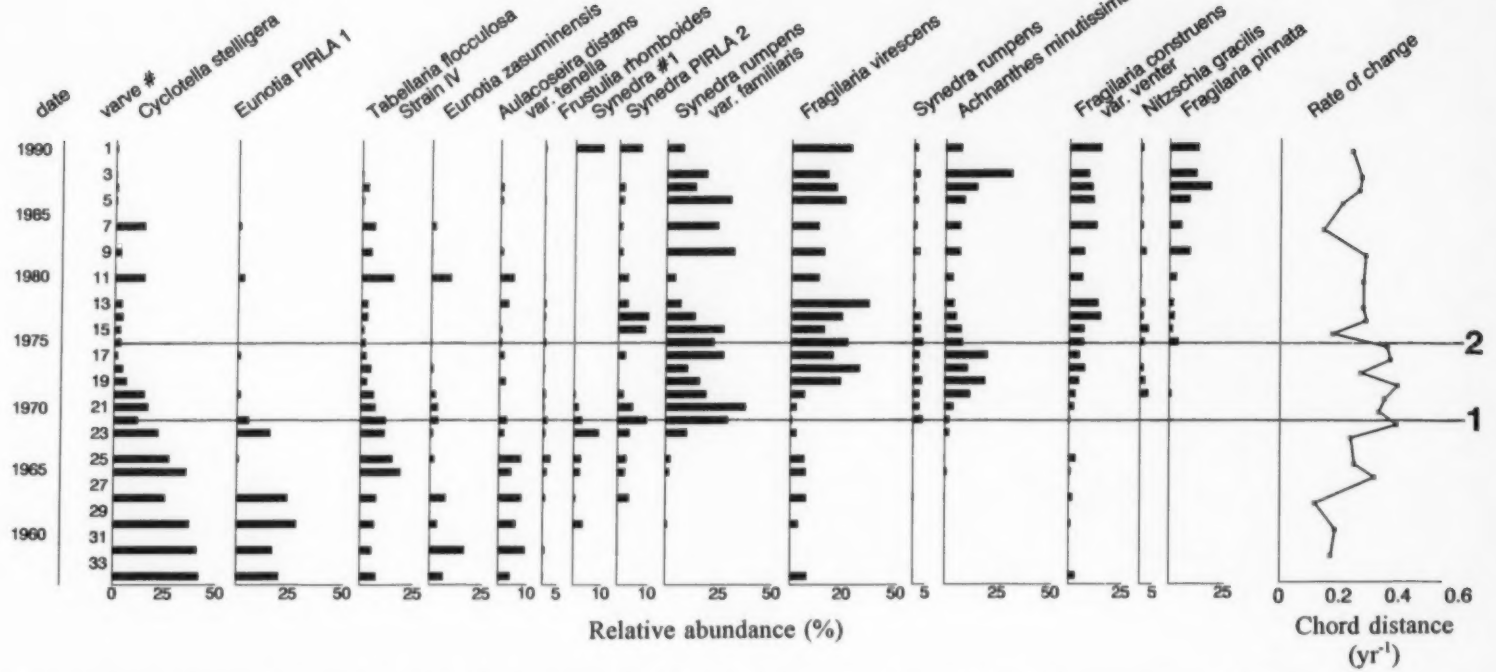

FIG. 1. Stratigraphic distributions (expressed as relative (%) abundances) and the rate of change (chord distance (yr^{-1})) of common diatoms in the sediments of Lake 227. 1 = start of experimental additions of nitrogen (N) and phosphorus (P) at a N:P of 13:1 (by mass); 2 = start of experimental additions of N and P at a N:P of 5:1 (by mass).

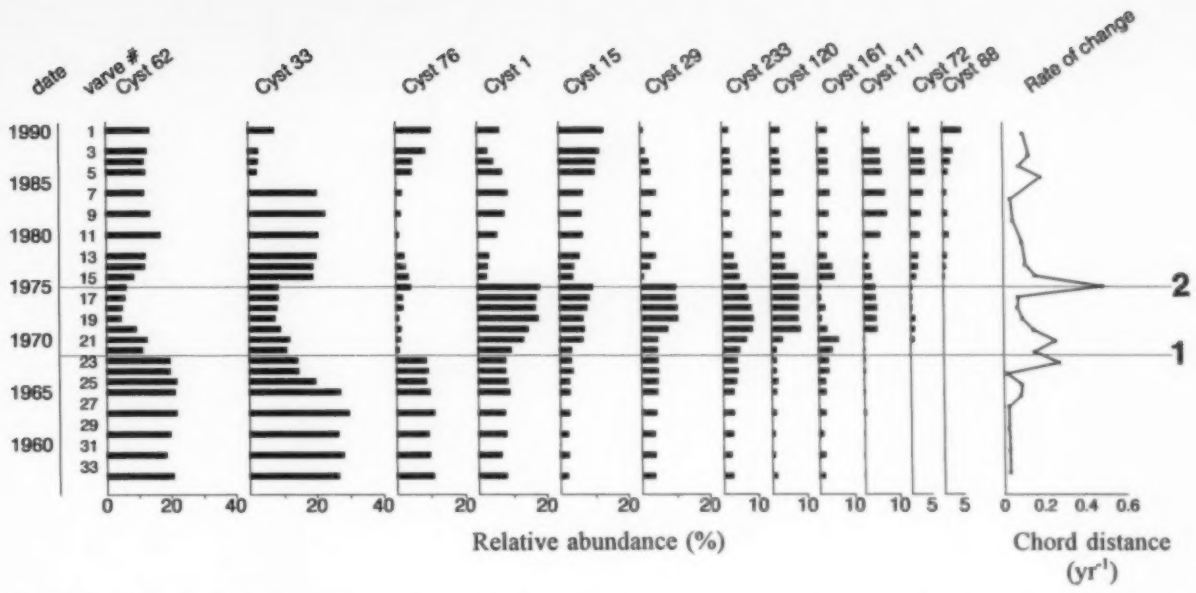

FIG. 2. Stratigraphic distributions (expressed as relative (%) abundances) and the rate of change (chord distance (yr^{-1})) of common chrysophycean cysts in the sediments of Lake 227. 1 = start of experimental additions of nitrogen (N) and phosphorus (P) at a N:P of 13:1 (by mass); 2 = start of experimental additions of N and P at a N:P of 5:1 (by mass).

Results and Discussion

Diatom valves (Fig. 1), chrysophyte cysts (Fig. 2), and chrysophyte scales (Fig. 3) were abundant and well-preserved in all samples. Fourteen diatom taxa showed relative abundances >2% of the fossil sum between 1957 and 1990 (Fig. 1). Forty-five chrysophycean cyst morphotypes were identified with SEM from the sediments, 33 of which had previously been observed and described by authors from the Paleoecological Environmental Assessment and Research Laboratory, Queen's University (PEARL). Twelve of the most common ones are presented in Fig. 2. Scales from 16 chrysophyte species and one collective group (encompassing several small *Mallomonas* taxa, designated "*Mallomonas* small") were identified; the most common 10 taxa are presented in Fig. 3 using two methods of calculation (Siver 1991a), which revealed similar trends.

Assemblage Changes

All microfossil assemblages showed striking changes coinciding with the initial input of nutrients into the lake in 1969 (Figs. 1–3). The diatom and chrysophyte assemblages present prior to ca. 1969 are referred to as "premanipulation" assemblages, whereas those occurring after this time are referred to as "postmanipulation" assemblages.

Following an experimental decrease in the N:P ratios from 13:1 to 5:1 in 1975 (Leavitt et al. 1994), a second assemblage shift occurred in the cyst and scale stratigraphies (Figs. 2, 3). Diatom species composition changed less dramatically during this period, although a brief assemblage shift occurred ca. 1980 (Fig. 1). Both chrysophyte scale and cyst assemblages again revealed some species shifts near the top of the core, ca. 1986 (Figs. 2, 3).

There is considerable variation in the patterns of the estimated rates of change for the three algal groups (Figs. 1–3). There are higher inherent differences in rates of change between adjacent samples in the diatom (Fig. 1) and chrysophyte scale (Fig. 3) assemblages than in the chrysophyte cysts (Fig. 2). The maximum rates of change in the cysts coincide almost precisely with the start of addition of N and P at N:P = 13:1 (by mass) (Fig. 2, line 1) and the switch to an N:P = 5:1 (Fig. 2, line 2), whereas the peaks in the rate of change in the scale assemblages (Fig. 3) were delayed by 1–2 yr after these two manipulations. Relationships between the rates of change in the diatom assemblages and the experimental manipulations were less clear. The maximum rate of change occurred ~2–3 yr after the 1969 manipulation and was maintained for a further 3 yr until the 1975 manipulation. After the second manipulation, there was a consistently high rate of change for ~6–7 yr.

General patterns in the rate-of-change profiles suggested different responses by the three groups. Prior to manipulation, the diatoms showed a high rate of change from sample to sample, whereas the cysts showed a low rate of change. After manipulation, lags of 2–3 yr appeared in responses by some of the diatoms and chrysophyte scales, but no lag was evident in the response by the cyst assemblages.

Premanipulation Assemblages (ca. 1957–1968)

There are no water chemistry data available for Lake 227 before 1968, so all environmental reconstructions before then must be based solely on paleolimnological inference.

Diatoms

From ca. 1957–1968, the diatom community was dominated (relative abundances >10%) by *Cyclotella stelligera* (Cl. and Grun.) V.H. (1957–1969), *Tabellaria flocculosa* (Roth) Kütz. Strain IV sensu Koppen (1965–1966), *Aulacoseira distans* var. *tenella* (Nygaard) Florin Simonsen (1959), *Eunotia* PIRLA 1 (1957–1963), and *E. zasuminensis*

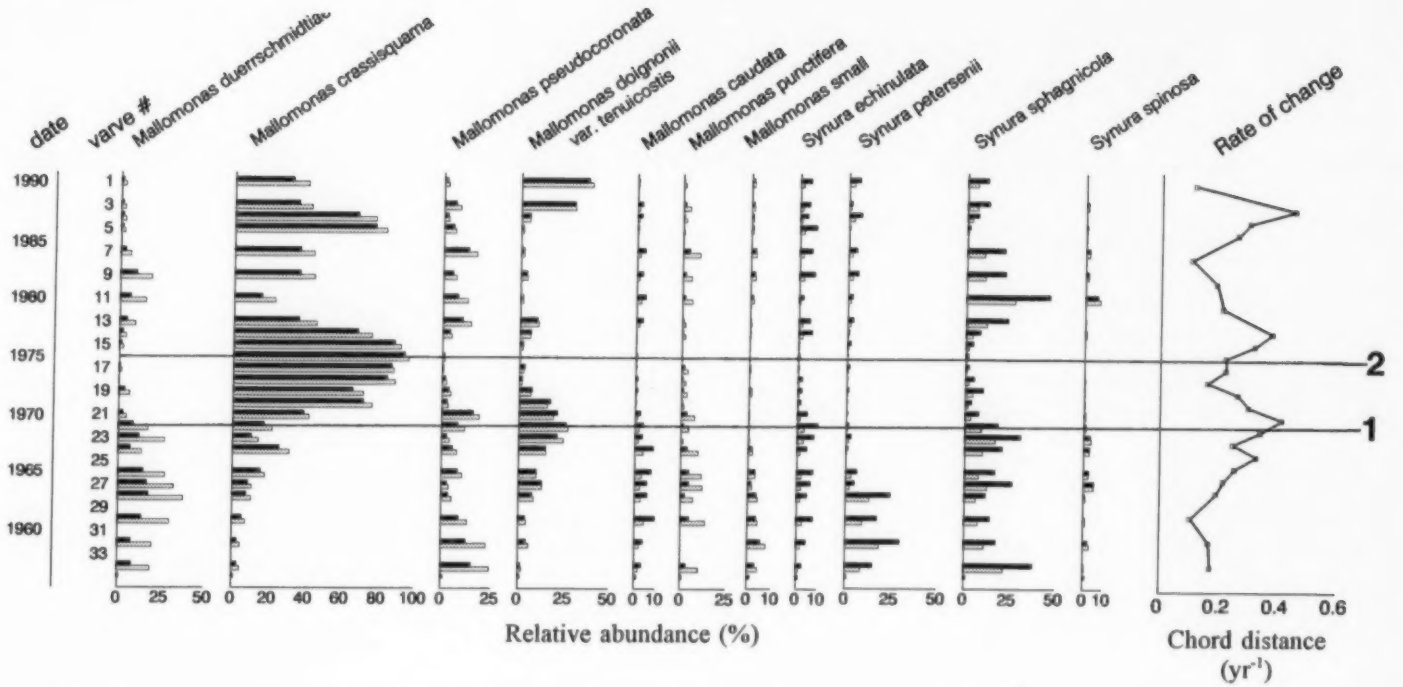

FIG. 3. Stratigraphic distributions (expressed as relative (%) abundances) and rate of change (chord distance (yr^{-1})) of common chrysophycean scales in the sediments of Lake 227. Solid bars are simple relative abundances (%) of scales; hatched bars are scale counts that have been proportionally adjusted to the number of scales per individual chrysophyte species. 1 = start of experimental additions of nitrogen (N) and phosphorus (P) at a N:P of 13:1 (by mass); 2 = start of experimental additions of N and P at a N:P of 5:1 (by mass).

(Cab.) Korner (1959). All taxa are characteristic of unproductive, slightly acidic waters. For example, many *Eunotia* species are restricted to acidic waters (Bourelly 1968). Nicholls and Carney (1979) found the planktonic *Eunotia zasuminensis* in unproductive northern Ontario lakes (total P [TP] = 8–25 $\mu g \cdot L^{-1}$, $n = 7$ lakes), and poorly buffered lakes with low alkalinity (4-5 mg $CaCO_3 \cdot L^{-1}$, $n = 7$ lakes). *Eunotia* PIRLA 1 is a finely striated planktonic taxon that has a high length to breadth ratio that was documented in the PIRLA iconograph (Camburn et al. 1984–1986). Relatively few autecological data are available for this taxon because *Eunotia* PIRLA 1 was present, in low abundance, in only five Adirondack lakes (pH 4.88–7.75).

Aulacoseira distans var. *tenella* is an acidophilic taxon (Camburn and Kingston 1986) that was recorded in 36 Adirondack lakes with a pH range of 4.88–7.7; it occurred at maximum abundance (23.9%) in Rock Pond (pH = 6.85). It was also found in 14 lakes from the PIRLA Northern Great Lakes Region (Kingston et al. 1990; relative abundance = 0.2–26.4%) that had pH values ranging from 5.21–7.25.

Tabellaria flocculosa Strain IV is an epiphytic taxon (Koppen 1975). Stockner (1971) found it in the littoral zone of ELA lakes in the spring. However, it was dispersed by turbulence into the plankton by June. Findlay and Shearer (1992) recorded it as a dominant taxon in 35 unmanipulated ELA lakes, and they classified it as acidophilic. It was also sometimes common (maximum relative abundance = 11.2%) in 48 of 98 PIRLA Adirondack lakes sampled (pH = 4.48–7.39).

Cyclotella stelligera is commonly found in unproductive to moderately productive systems (Schindler and Holmgren 1971; Stoermer et al. 1985; Christie 1993). It appears to be a pH-indifferent taxon (Dixit et al. 1991; Findlay and Shearer 1992). It was found in lakes located on the Canadian Shield (Dixit et al. 1991; Findlay and Shearer 1992), in alkaline lakes from southeastern Ontario (Christie 1993), and in the Laurentian Great Lakes (Stoermer et al. 1985).

Cysts

Shifts in the relative percentages of chrysophyte cyst morphotypes were slight (i.e., <5% change) in the premanipulation sediments, presumably indicating fairly stable lake conditions during this time. Cysts 62 and 76 (Duff and Smol 1991), 33 (Duff and Smol 1989), and 1 (Duff and Smol 1988) dominated, collectively accounting for 75% of the premanipulation cyst assemblage.

Cysts 62, 33, and 76 are highly ornamented taxa typical of unproductive systems. Cyst 33 was described originally from an oligotrophic, mid-arctic lake where it briefly dominated the chrysophyte assemblage (Duff and Smol 1989). Cysts 62 and 76, originally described from Adirondack Park, New York, were also found in oligotrophic and somewhat acidic waters (Duff and Smol 1991).

Cyst 1 is a small, unornamented cyst lacking a collar. It has been described from a number of temperate and arctic environments (Duff and Smol 1988, 1991; Zeeb et al. 1990; Rybak et al. 1991). It appears to have a wide ecological tolerance and is pH-indifferent (Rybak et al. 1991), but may occur in more productive waters than the previously discussed taxa (Zeeb et al. 1990).

Scales

Premanipulation scales were dominated by a relatively diverse assemblage of *Mallomonas duerrschmidtiae* Siver, Hamer and Kling, *M. pseudocoronata* Prescott, *Synura petersenii* Korshikov, and *S. sphagnicola* (Korsh.) Korshikov. *Mallomonas duerrschmidtiae* is found over relatively narrow environmental gradients, almost exclusively occurring in lakes of pH <7 (AWM = 5.76) that are low in specific conductance and TP (Siver 1991b).

Mallomonas pseudocoronata is a more cosmopolitan species. It is found over a wide TP gradient (<10 – >35 $\mu g \cdot L^{-1}$), specific conductance (21–170 μS), and pH values (5.75–8.75; AWM = 7.72) (Siver 1991b).

Synura petersenii is an environmental generalist; it is the most commonly reported *Synura* species in the world. *Synura sphagnicola* is reported as occurring predominantly in acidic waters; it has often been found in large populations in acid bog lakes (Kristiansen 1975; Dürrschmidt 1980; Roijackers and Kessels 1986).

Postmanipulation Assemblages (1969–1989)

From 1969 until 1989, annual nutrient additions to Lake 227 resulted in increased annual averages of TP (from 1–8 $\mu g \cdot L^{-1}$ in 1970; up to 14 $\mu g \cdot L^{-1}$ in 1971) and total N (TN) concentrations (from 245 $\mu g \cdot L^{-1}$ in 1969 to >600 $\mu g \cdot L^{-1}$ in 1970) in the water column. pH values increased (>9.0) in the epilimnion during the ice-free season (Schindler et al. 1980).

Diatoms

Numerous lacustrine studies have shown that diatom assemblages respond to changes in nutrient concentrations (Brugam 1978; Engstrom et al. 1985; Anderson 1989) and to pH and pH-related factors such as alkalinity (Charles et al. 1989). Therefore, it is likely that changes in the postmanipulation diatom assemblages observed in Lake 227 (Fig. 1) may be related to both increased nutrient concentrations and increased pH.

The relative abundances of acidophilic and generalist diatoms declined after N and P were added in 1969 (Fig. 1). Relative abundances of *Cyclotella stelligera* and the acidophilic *Eunotia* PIRLA 1, *E. zasuminensis*, *Tabellaria flocculosa* Strain IV, and *Aulacoseira distans* var. *tenella* decreased and were either low or zero for the remainder of the core. The decline of the four acidophilic taxa may be related to the increased pH (8–9) in the epilimnion from July 1969 until October 1969 (Schindler et al. 1971). *Cyclotella stelligera* is a pH-indifferent taxon that is moderately tolerant of increased nutrient enrichment (Stoermer et al. 1985), which is consistent with its persistence in modest numbers following the manipulation.

The acidophilic taxa were replaced by *Synedra* PIRLA 2, *S. rumpens* var. *familiaris* (Kütz) Hust., and *S. rumpens* Kütz following fertilization (Fig. 1). Many researchers have observed increased abundances of *Synedra* taxa in response to accelerated anthropogenic nutrient loading (e.g., Lake Washington: Stockner and Benson 1967; Linsley Pond: Brugam 1978; Found Lake: Smol and Dickman 1981; Little Round Lake: Smol et al. 1983). Increased abundances of *Synedra* taxa were also observed following liming events, presumably in response to increased pH levels (Loch Fleet: Flower et al. 1990; Lysevatten: Renberg and Hultberg 1992). Although the taxonomy of *Synedra* has been in flux, increases in spindle-shaped *Synedra* taxa appear to be typical of the early stages of nutrient enrichment or increased pH levels.

The ecological characteristics of the three dominant *Synedra* taxa recorded in Lake 227 have been discussed in recent studies. For example, Findlay and Shearer (1992) found *S. rumpens* in 12 of 36 unmanipulated lakes at the ELA and they classified it as pH indifferent. The PIRLA database showed that *Synedra rumpens* and *S. rumpens* var. *familiaris* were not common at any sites in the Adirondacks or from the northern Great Lake States, so it is difficult to establish pH tolerances for these taxa.

Synedra PIRLA 2 was common in 28 Adirondack lakes that had a pH range of 5.1–7.77. Its maximum abundance (24.7%) was recorded in Clear Pond, an unproductive lake (TP = 1 $\mu g \cdot L^{-1}$: chlorophyll *a* = 0.3 $\mu g \cdot L^{-1}$) with a pH of 7.05. However, it was also abundant (14.2%) in Mt. Arab Pond, a more productive system (TP = 15.5 $\mu g \cdot L^{-1}$) with a pH of 6.94.

From 1970–1988, the sedimentary diatom assemblage continued to be dominated by *Synedra rumpens* var. *familiaris*; however, increased abundances of benthic taxa, such as *Fragilaria virescens* Ralfs, *F. construens* var. *venter* (Ehrenb.) Grun., *Achnanthes minutissima* Kütz, and *Nitzschia gracilis* Hust., were also observed (Fig. 1). The relative abundance of *F. pinnata* Ehrenb. also increased in 1974. These benthic diatoms were clearly more competitive following the 1969 manipulation, which elevated nutrient and pH levels. These taxa would have been tolerant of the increased pH that characterized Lake 227 during the growing seasons following fertilization, and they have often been classified as circumneutral taxa (*Achnanthes minutissima*: Findlay and Shearer 1992) or alkaliphilic taxa (*F. construens* var. *venter*: Brugam 1983; *F. pinnata*: Findlay and Shearer 1992).

Cysts

Between 1969–1975, cysts 62, 33, 76, and 161 (Zeeb and Smol 1993) decreased in relative abundance, whereas cysts 1, 15 (Duff and Smol 1988), 29 (Duff and Smol 1989), 233 (this paper), 120 (Duff and Smol, in Duff et al. 1992), and 111 (Zeeb et al. 1990) increased. Also, cyst 72 (Duff and Smol 1991) was observed for the first time in the core.

The increase in cyst 1 (from <10% in 1968 to >15% in 1970) coincided with the 1969 nutrient additions to Lake 227, and is consistent with its increase in Little Round Lake, Ontario, following eutrophication after land clearance (Zeeb et al. 1990). Cyst 1 is similar to those produced by *Paraphysomonas* spp. (Preisig and Hibberd 1982, 1983), taxa that are common in the eutrophic waters of Lake Tystrup, Denmark (Kristiansen 1985).

Cysts 15 and 29, which also increased in abundance ca. 1969–1970, are morphologically similar to cyst 1; cyst 15 is about three times larger in diameter, and cyst 29 is distinguished by a shallow conical pore. Both cysts have been observed in arctic (Duff and Smol 1988) and temperate (Zeeb et al. 1990; Zeeb and Smol 1993) regions. Cyst 29 was found in the early postglacial sediments of Elk Lake, Minnesota, but peaked in abundance near the top of that core when nutrient conditions and temperatures were higher (Zeeb and Smol 1993).

Cyst 233, described for the first time in Appendix 1, increased from premanipulation values of ca. 4% to postmanipulation values of 10%. Morphologically, it is most similar to cyst 41, described from a mid-arctic lake (Duff and Smol 1989), and cyst 78 from Adirondack Park (Duff and Smol 1991). Cysts 41, 78, and 233 are spherical with a hooked collar, which is typical of the cyst produced by *Dinobryon cylindricum* Imhof (Sandgren 1980a, 1983; Donaldson and Stein 1984). Cysts with hooked collars are often recorded in cyst assemblages (e.g., Adam and Mahood (1979) type 56; *cysta curvicollis* Nygaard (Rybak 1986); Rybak et al. (1987) cyst 3; Sandgren and Carney (1983) cyst 9), and are usually associated with oligotrophic to mildly eutrophic conditions (Rybak 1986; Rybak et al. 1987; Carney and Sandgren 1983).

Cyst 120 is a larger form of cyst 29 and was described from high-arctic ponds (Duff et al. 1992). It has previously been found in circumneutral to alkaline waters of low productivity (Duff et al. 1992; Zeeb and Smol 1993; Rybak et al. 1987). This study suggests that cyst 120 is also present in high productivity environments.

Cyst 161 peaked in abundance ca. 1970, but then dropped to lower values for the next five years. It is probably produced by *Dinobryon divergens* Imhof (Sheath et al. 1975; Sandgren 1980b), which is a pH-indifferent to alkaliphilic species that thrives in mesotrophic to eutrophic waters (Eloranta 1989).

Cyst 111 increased from trace levels in the premanipulation assemblage to ca. 4% in 1971. This cyst is produced by *Spiniferomonas trioralis* Takahashi, which is a cosmopolitan species found across a wide range of environmental conditions (e.g., Dürrschmidt 1980; Nicholls 1981; Roijackers and Kessels 1981; Croome and Tyler 1988; Siver 1988). It is predominantly a spring-blooming taxon (Dürrschmidt 1980; Kristiansen 1988), and cyst formation occurs during, or prior to, spring ice break-up (Skogstad 1986). Previous paleolimnological reports have linked this cyst with eutrophic conditions (Nygaard 1956; Rybak 1986; Carney and Sandgren 1983; Zeeb et al. 1990), which is consistent with our observations in Lake 227.

Scales

Immediately following the 1969 lake manipulation, *Mallomonas crassisquama* (Asmund) Fott increased dramatically (from <20% to >80% abundance). *Mallomonas crassisquama* is an environmental generalist found over wide ranges of pH, specific conductance, TP, and temperature (Siver 1991b). A less striking increase occurred with *M. doignonii* var. *tenuicostis* Asmund and Cronberg, but it had declined by 1972. Very few ecological data are available for *M. doignonii*, but it is believed to be a cold-water organism, tentatively classified as acidophilic, and usually present in habitats with specific conductance levels <53 μS (Siver 1991b). Increased abundances of the above two taxa were mirrored by decreased abundances of *M. duerrschmidtiae*, *Synura petersenii*, small *Mallomonas* species, and *M. caudata* Ivanov. The ecological requirements of *M. caudata* are similar to those of M. *crassisquama* (Siver 1991b).

Postmanipulation Change in the N:P Ratio (1976–1990)

The second major chrysophyte assemblage shift occurred ca. 1976, which followed the experimental change in N:P ratios from 13:1 to 5:1 by mass in 1975 (Figs. 2, 3). During 1975, N additions were reduced, and N_2-fixing cyanobacteria increased in abundance and largely replaced the chlorophytes (Leavitt and Findlay 1994). During this period, total dissolved nitrogen (TDN) continued to rise (up to 750 $\mu g \cdot L^{-1}$ in 1989); total dissolved phosphorus (TDP) remained fairly steady (1983: 13 $\mu g \cdot L^{-1}$; 1985: 11 $\mu g \cdot L^{-1}$)

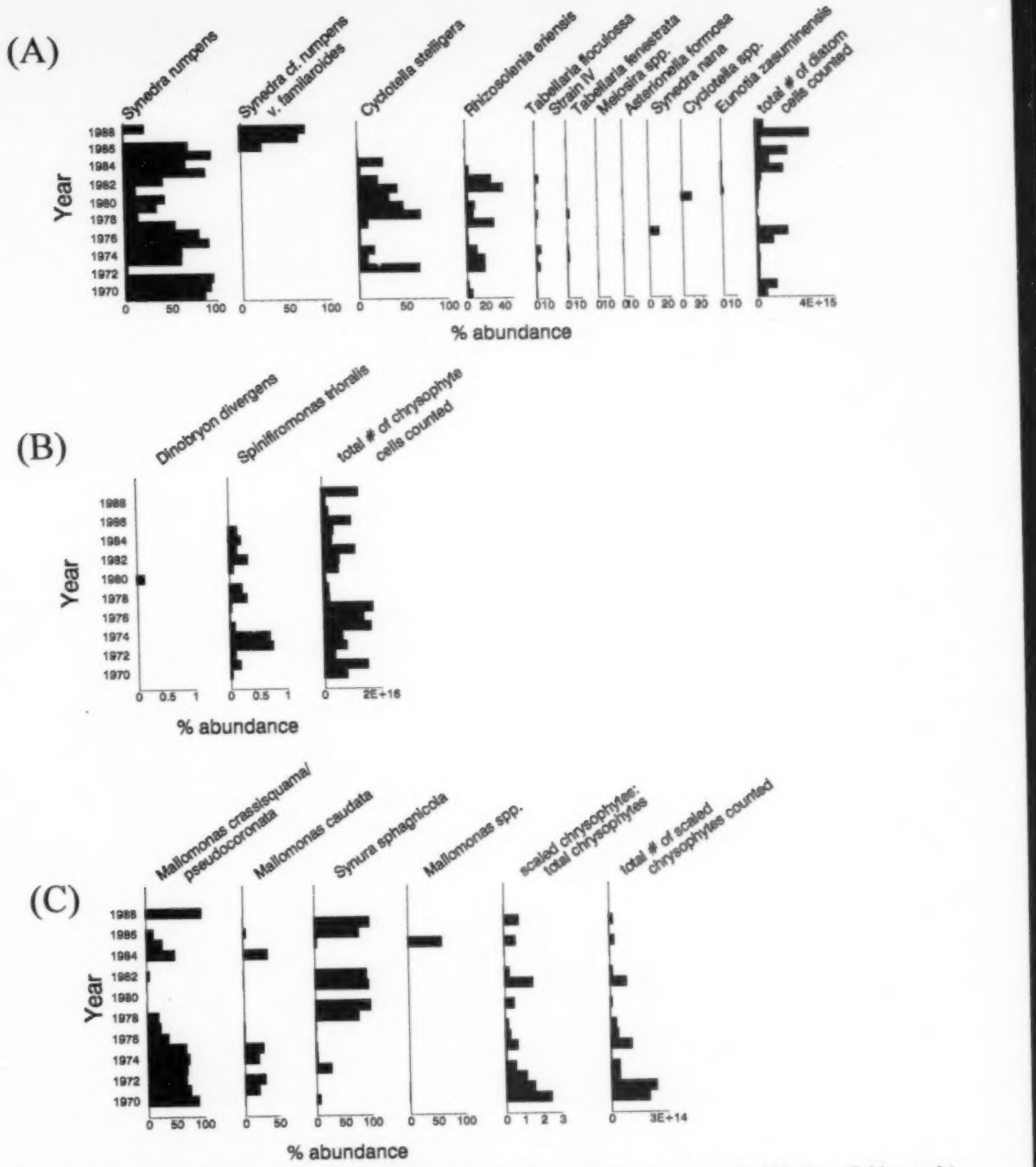

FIG. 4. Percent abundances and number of cells counted of selected phytoplankton species in Lake 227 (from field records). (A) Diatoms. (B) Chrysophytes. (C) Scaled chrysophytes.

but peaked at 29 $\mu g \cdot L^{-1}$ in 1984. Following the change in N:P ratios in 1975, relative abundance of cysts 62 and 33 increased to almost premanipulation levels. Relative abundances of cysts 72 and 88 (Duff and Smol 1991) also increased, whereas cysts 1, 15, 29, 233, and 120 declined. The premanipulation and post–1975 cyst assemblages were similar, although the latter was more diverse; both assemblages were dominated by highly ornamented taxa. Unlike chrysophyte assemblages, diatom communities showed little response to the change in the N:P ratio.

A less striking shift in cyst assemblage occurred ca. 1985. The relative abundance of cyst 62 was unaffected, but cyst

33 decreased from >20% to <5%, and was replaced by cysts 76 and 15. This shift may have been linked to a sudden increase in TDP (from 14 $\mu g \cdot L^{-1}$ in 1983 to 29 $\mu g \cdot L^{-1}$ in 1984) (D.L. Findlay and H.J. Kling, unpublished data).

The scaled chrysophytes also shifted with the change in nutrient ratios ca. 1976. Relative abundances of *M. crassisquama* and *M. doignonii* var. *tenuicostis* scales decreased, whereas those of *Synura sphagnicola* increased (Fig. 3). However, *M. crassisquama* and *M. doignonii* var. *tenuicostis* eventually recovered to premanipulation values (Fig. 3).

Variance Partitioning of the Algal Groups

The stratigraphic changes in algal assemblages described above were related to changes in water chemistry by using a series of partially constrained ordination models. The overall amount of variation explained by water chemistry and time was 79.1%, 75.0%, and 95.7% for diatoms, scales, and cysts, respectively. Chemical changes independent of time explained 60.5%, 66.3%, and 50.0% of the variation, whereas the time data independent of water chemistry explained only 6.3%, 5.9%, and 5.1% of the variation. The small amount of total variation accounted for by time alone indicated that no fundamental, temporally structured, environmental process had been missed. Moreover, the analyses indicated that there was a strong relationship between changes in water chemistry and the algal assemblages. Furthermore, the low incidence of unexplained variation in chrysophyte cysts (4.3%) suggested that there was a very close relationship between cysts and water chemistry in Lake 227 between 1969 and 1989.

Comparison of Fossil and Planktonic Diatoms and Chrysophytes

Diatoms

The planktonic diatom assemblage was dominated (i.e., relative abundance >20%) by either *Synedra rumpens* (1970–1972; 1974–1978; 1983–1987), *Cyclotella stelligera* (1973; 1979–1982; 1985), *Rhizosolenia eriensis* H.L. Smith (1973–1974; 1978; 1982–1983), or *Synedra* cf. *rumpens* var. *familaroides* Grun. (1987–1989), or a combination of these taxa in different years from 1970 until 1988 (Fig. 4A). *Synedra rumpens* probably represented a grouping of the three main *Synedra* taxa that were detected in the sediment core (i.e., *Synedra rumpens*, *S. rumpens* var. *familiaris*, and *Synedra* PIRLA 2; Fig. 1). In fact, *S.* cf. *rumpens* var. *familaroides*, observed in 1988 (Fig. 4A), was probably synonymous with *Synedra* PIRLA 2 recorded from the sediment core (Fig. 1).

The relative abundance of *Cyclotella stelligera* increased in the phytoplankton assemblage (Fig. 4A), whereas it decreased in the sediment core (Fig. 1). The decline may be the result of an influx of benthic taxa that were both tolerant of the increased pH and capable of using the increased nutrients available following fertilization (as discussed above). These benthic taxa would not have been collected in the water samples analyzed for phytoplankton, but they would be observed in the integrated sediment samples.

Rhizosolenia eriensis was common in the phytoplankton assemblage (Fig. 4A). However, its valves were probably destroyed during the sediment analyses by the H_2SO_4 used to prepare the samples (Yung et al. 1988).

Chrysophytes

Thirty chrysophyte species, six genera, and two collective groups (i.e., "small" and "large" chrysophytes) were differentiated in the phytoplankton counts from Lake 227 (D.L. Findlay and H.J. Kling, unpublished data). Only two of our common sedimentary cyst morphotypes could be linked to chrysophyte species. Cyst 111 (*Spiniferomonas trioralis*) was present throughout the sediment core, but started to increase in abundance ca. 1969 and peaked ca. 1973 (Fig. 2). Phytoplankton records showed a similar trend; maximum numbers of this species were observed in 1973 (ca. 0.8% abundance), but it was absent in the phytoplankton samples from 1986 onwards (Fig. 4B).

Cyst 161 (probably *Dinobryon divergens*) was also present throughout the sediment core. It peaked ca. 1970 and was more abundant in the upper part of the core (1976–1990) than in the lower part (Fig. 2). This species was only recorded in the phytoplankton in 1980 in very low abundances (<1.0%; Fig. 4B).

Mallomonas crassisquama/*M. pseudocoronata* were abundant in the phytoplankton in 1970, and numbers remained high until ca. 1975 (Fig. 4C). These two taxa were always lumped together with *M. duerrschmidtiae* in the phytoplankton counts because the three taxa were not separated taxonomically until 1990 (Siver et al. 1990). Unfortunately, although *M. crassisquama* and *M. pseudocoronata* have similar ecological tolerances (except the former has a wider pH range), *M. duerrschmidtiae* has very different nutrient and pH optima (Siver 1991b). Important ecological information was, therefore, lost by lumping the three taxa together.

Mallomonas doignonii var. *tenuicostis*, another dominant species in the sediment chrysophyte assemblage (Fig. 3), was either not identified in the phytoplankton counts or it was lumped into the "*Mallomonas* spp." category (Fig. 4C). The presence of *M. caudata* and the increased abundance of *Synura sphagnicola* from ca. 1978–1988 in the sediment core (Fig. 3) was reflected in the phytoplankton records (Fig. 4C).

It is difficult to compare the paleolimnological data to the annual phytoplankton records for Lake 227 because of taxonomic inconsistencies. For example, phytoplankton samples were fixed with Lugol's solution and counted using a modified Utermöhl method (Nauwerck 1963). The identification of the diatom taxa in these samples probably is reliable only to the genus level because specific or subspecific identification of many taxa depends upon ornamentation of the cell wall, which often requires high-resolution microscopy. Furthermore, it is important to note that the phytoplankton collections represent discrete samples, whereas lake sediments record algal assemblages integrated over time (365 days of the year) and space (from the entire water column and from various habitats).

Conclusions

In view of the fact that no water chemistry data were collected before manipulation, the paleolimnological data provide important baseline information for Lake 227. The lake was unproductive (pH ~6) before nutrient addition, as evidenced by the dominant taxa in the premanipulation diatom assemblage: *Cyclotella stelligera*, *Tabellaria flocculosa* Strain IV, *Aulacoseira distans* var. *tenella*, *Eunotia* PIRLA 1, and *E. zasuminensis*. Chrysophyte cyst and scale assemblages were

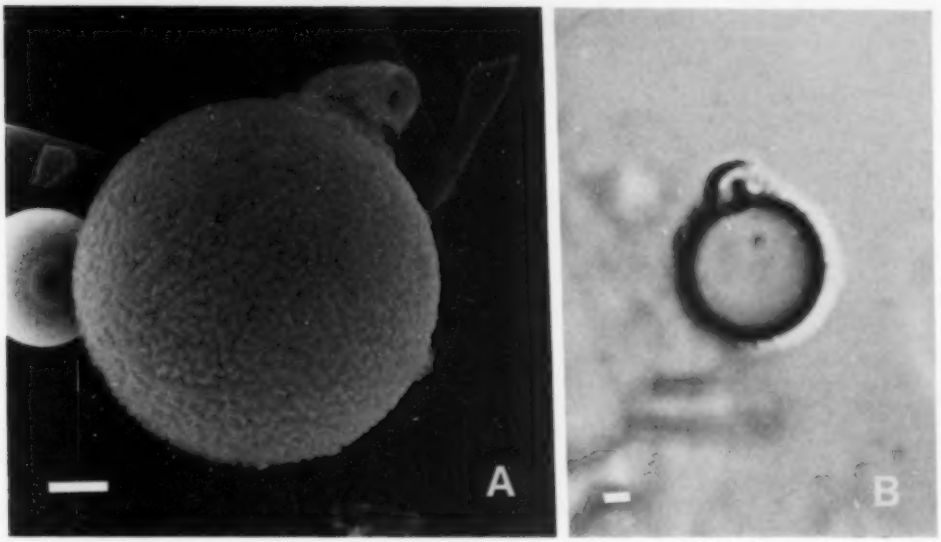

FIG. A1. Stomatocyst 233 (Zeeb and Smol), a common cyst morphotype found in the sediments of Lake 227. (A) Scanning electron micrograph. (B) Light micrograph. Scale bars = 2 μm.

diverse and relatively constant during this period. Although there were some subtle species changes before 1969, these natural fluctuations were insignificant compared with the striking changes that occurred after fertilization started.

Stratigraphic changes in diatom and chrysophyte assemblages were clearly related to increased nutrient availability and/or increased pH resulting from lake fertilization. Moreover, there was no significant lag period between initial lake manipulation and when changes were first observed in sedimentary algal assemblages.

The increase in epilimnetic nutrient concentrations and pH after 1969 coincided with decreased relative abundance of the premanipulation assemblage, in conjunction with large increases in the relative abundances of *Synedra* PIRLA 2, *S. rumpens* var. *familiaris*, *Mallomonas crassisquama*, and cysts 1, 15, 29, 120, and 233. From 1970 until the present, the diatom assemblage has continued to be dominated by alkaliphilic or pH-indifferent taxa. Chrysophyte populations had a second large assemblage shift ca. 1976, immediately following the change in N:P ratio, characterized by a decrease in *Mallomonas crassisquama*, an increase in *Synura sphagnicola*, and a return of dominant premanipulation cysts.

It was difficult to compare the paleolimnological data with the annual phytoplankton records that have been maintained for Lake 227, primarily because of taxonomic problems. Furthermore, more diatom and chrysophyte taxa were recorded in the sediment core than in the plankton samples. For example, scaled chrysophytes were rare in the overall phytoplankton records, but showed a striking response to nutrient addition in the sedimentary profiles.

In summary, the paleolimnological data presented in this study complement the annual phytoplankton records presently available and provide additional information describing the spatial and temporal responses of diatom and chrysophyte assemblages to whole-lake fertilization. The data also provide insights into the relative responsiveness of different algal groups to major environmental perturbations.

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Appendix

Description of a new Chrysophyte Cyst

Following the International Statospore Working Group (ISWG) guidelines (Cronberg and Sandgren 1986), we describe a new cyst morphotype that was common in the Lake 227 sediments. The number in parentheses following the authors' names indicates the number of scanning electron microscope (SEM) micrographs on which the description is based. The negative number, locality information, and first figure refer to the characteristic SEM specimen. Cyst numbering follows consecutively from Duff and Smol (1994).

Stomatocyst 233, Zeeb and Smol (6)
Negative No.: J.P. Smol 586, Plate 1
Locality: Experimental Lakes Area Lake 227, Ontario, varve No. 17

This is a large, spherical stomatocyst (diam. 12.3–14.4 μm). The collar has a conical base with a gradual basal margin, and a hooked apical portion (basal diam. ca. 3.6 μm; apical diam. 1.6–2.6 μm; height to flexure 2.8–3.2 μm; length to 5.5 μm). The collar is radially striated. The entire cyst surface is densely scabrate with occasional smooth patches. The scabrae may be elongated (diam. 0.2–0.4 μm; height 0.2–0.3 μm) and extend up the length of the collar. This stomatocyst is very similar to one produced by *Dinobryon cylindricum* Imhof (Donaldson and Stein 1984; Sandgren 1980a, 1983, 1989),

although scabrate forms have not been recorded. With SEM and light microscopy, stomatocyst 233 can be distinguished from stomatocyst 41 (Duff and Smol 1989) by the presence of scabrae, and from stomatocyst 78 (Duff and Smol 1991) by the absence of a bipartite collar and the lack of verrucae.

References

ADAM, D.P., AND A.D. MAHOOD. 1979. Modern and Holocene chrysommonad cysts from Alta Morris Lake, Eldorado County, California. US Geol. Surv. Open-File Rep. 70–1461. 13 p.

ANDERSON, N.J. 1989. A whole-basin diatom accumulation rate for a small eutrophic lake in northern Ireland and its palaeoecological implications. J. Ecol. 11: 926–946.

ASMUND, B., AND J. KRISTIANSEN. 1986. The genus *Mallomonas* (Chrysophyceae). Opera Bot. 85: 128 p.

BENNETT, K.D., S. BOREHAM, M.J. SHARP, AND V.R. SWITSUR. 1992. Holocene history of environment, vegetation and human settlement on Catta Ness, Lunnastung, Shetland. J. Ecol. 80: 241–273.

BORCARD, D., P. LEGENDRE, AND P. DRAPEAU. 1992. Partialling out the spatial component of ecological variation. Ecology, 73: 1045–1055.

BOURELLY, P. 1968. Les algues d'eau douce. Tome II. Les algues jaunes et brunes. N. Boubee et Cie, Paris.

BRUGAM, R.B. 1978. Human disturbance and the historical development of Linsley Pond. Ecology, 59: 19–36.

BRUGAM, R.B. 1983. The relationship between fossil diatom assemblages and limnological conditions. Hydrobiologia, 98: 223–235.

CAMBURN, K.E., AND J.C. KINGSTON. 1986. The genus *Melosira* from soft-water lakes with special reference to northern Michigan, Wisconsin and Minnesota, p. 17–34. *In* J.P. Smol et al. [ed.] Diatoms and lake acidity. Dr. W. Junk Publishers, Dordrecht, The Netherlands.

CAMBURN, K.E., J.C. KINGSTON, AND D.F. CHARLES [ed.]. 1984–1986. PIRLA diatom iconograph. PIRLA Unpubl. Rep. Ser. 3. Indiana University, Bloomington, IN.

CARNEY, H.J., AND C.D. SANDGREN. 1983. Chrysophycean cysts: indicators of eutrophication in the recent sediments of Frains Lake, Michigan, U.S.A. Hydrobiologia, 101: 195–202.

CARNEY, H.J., M.C. WHITING, K.E. DUFF, AND D.R. WHITEHEAD. 1992. Chrysophycean cysts in Sierra Nevada (California) lake sediments: paleoecological potential. J. Paleolimnol. 7: 73–94.

CHARLES, D.F., R.W. BATTARBEE, I. RENBERG, H. VAN DAM, AND J.P. SMOL. 1989. Paleoecological analyses of lake acidification trends in North America and Europe using diatoms and chrysophytes, p. 207–270. *In* S.A. Norton, S.E. Lindberg, and A.L. Page [ed.] Acidic precipitation volume 4: soils, aquatic processes and lake acidification. Springer-Verlag, Stuttgart.

CHARLES, D.F., M.W. BINFORD, E.T. FURLONG, R.A. HITES, M.J. MITCHELL, S.A. NORTON, F. OLDFIELD, M.J. PATERSON, J.P. SMOL, A.J. UUTALA, J.R. WHITE, D.R. WHITEHEAD, AND R.J. WISE. 1990. Paleoecological investigation of recent lake acidification in the Adirondack Mountains, N.Y. J. Paleolimnol. 3: 195–241.

CHRISTIE, C. 1993. Paleoecological reconstruction of lake trophic status: the effect of human activity on lake conditions in southeastern Ontario in the recent (ca. 200 years) past. Ph.D. thesis, Queens University, Kingston, Ont. 211 p.

CLEVE-EULER, A. 1951–1955. Die Diatomeen von Schweden und Finnland. Kungl. Svenska Vetenskapsakademiens Handlingar. Fjarde Serien Band 2(1), Band 3(3), Band 4(5). (Republished by J. Cramer, Berlin, as Bibl. Phycol. 5, 1968).

CRONBERG, G., AND C.D. SANDGREN. 1986. A proposal for the development of standardized nomenclature and terminology for chrysophycean statospores, p. 317–328. *In* J. Kristiansen and R.A. Andersen [ed.] Chrysophytes: aspects and problems. Cambridge University Press, Cambridge, MA.

CROOME, R.L., AND P.A. TYLER. 1988. Phytoflagellates and their ecology in Tasmanian polyhumic lakes. Hydrobiologia, 161: 245–253.

DIXIT, S.S., A.S. DIXIT, AND J.P. SMOL. 1991. Multivariable environmental inferences based on diatom assemblages from Sudbury (Canada) lakes. Freshwater Biol. 26: 251–266.

DONALDSON, D.R., AND J.R. STEIN. 1984. Identification of planktonic Mallomonadaceae and other Chrysophyceae from selected lakes in the lower Fraser Valley, British Columbia, Canada. Can. J. Bot. 67: 525–539.

DUFF, K.E., AND J.P. SMOL. 1988. Chrysophycean stomatocysts from the post-glacial sediments of a High Arctic lake. Can. J. Bot. 66: 1117–1128.

DUFF, K.E., AND J.P. SMOL. 1989. Chrysophycean stomatocysts from the post-glacial sediments of Tasikutaaq Lake, Baffin Island, N.W.T. Can. J. Bot. 67: 1649–1656.

DUFF, K.E., AND J.P. SMOL. 1991. Morphological descriptions and stratigraphic distributions of the chrysophycean stomatocysts from a recently acidified lake (Adirondack Park, N.Y.). J. Paleolimnol. 5: 73–113.

DUFF, K.E., AND J.P. SMOL. 1994. Chrysophycean cyst flora from British Columbia (Canada) lakes. Nova Hedwigia, 58: 353–389.

DUFF, K.E., M.S.V. DOUGLAS, AND J.P. SMOL. 1992. Chrysophyte cysts in 36 Canadian high arctic ponds. Nord. J. Bot. 12: 471–499.

DÜRRSCHMIDT, M. 1980. Studies on the Chrysophyceae from Rio Cruces, Prov. Valdivia, south Chile by scanning and transmission microscopy. Nova Hedwigia 33: 353–388.

ELORANTA, P. 1989. On the ecology of the genus *Dinobryon* in Finnish lakes. Beih. Nova Hedwigia, 95: 99–109.

ENGSTROM, D.R., E.B. SWAIN, AND J.C. KINGSTON. 1985. A paleolimnological record of human disturbance from Harvey's Lake, Vermont: geochemistry, pigments and diatoms. Freshwater Biol. 15: 261–288.

FINDLAY, D.L., AND J.A. SHEARER. 1992. Relationships between sedimentary diatom assemblages and lakewater pH values in the Experimental Lakes Area. J. Paleolimnol. 7: 145–156.

FINDLAY, D.L., R.E. HECKY, L.L. HENDZEL, M.P. STAINTON, AND G.W. REGEHR. 1994. Relationship between N_2-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. Can. J. Fish. Aquat. Sci. 51: 2254–2266.

FLETT, R.J., D.W. SCHINDLER, R.D. HAMILTON, AND N.E.R. CAMPBELL. 1980. Nitrogen fixation in Canadian Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 37: 494–505.

FLOWER, R.J., N.G. CAMERON, N. ROSE, S.C. FRITZ, R. HARRIMAN, AND A.C. STEVENSON. 1990. Post-1970 water chemistry changes and paleolimnology of several acidified upland lakes in the U.K. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 327: 427–433.

GRIMM, E.C., AND G.L. JACOBSON. 1992. Fossil-pollen evidence for abrupt climate changes during the past 18 000 years in eastern North America. Clim. Dyn. 6: 179–184.

HUSTEDT, F. 1928–1966. Die Kieselagen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete. *In* Dr. L. Rabenhorst's Kryptogamenflora von Deutschland, Österreich und der Schweiz, parts 1–3. (Reprinted by Koeltz, Königstein, Germany, 1977).

HUSTEDT, F. 1930. Bacillariophyta (Diatomaceae). *In* A. Pascher [ed.] Die Süßwasserflora Mitteleuropas, Heft 10. Gustav Fischer Verlag, Jena.

KINGSTON, J.C., R.B. COOK, R.G. KREIS, JR., K.E. CAMBURN, S.A. NORTON, P.R. SWEETS, M.W. BINFORD, M.J. MITCHELL, S.C. SCHINDLER, L.C.K. SHANE, AND G.A. KING. 1990. Paleoecological investigation of recent lake acidification in the northern Great Lakes states. J. Paleolimnol. 4: 153–201.

KOPPEN, J.D. 1975. Morphological and taxonomic considerations of *Tabellaria* (Bacillariophyceae) from the north central United States. J. Phycol. 2: 236–244.

KRAMMER, K., AND H. LANGE-BERTALOT. 1986. Naviculaceae. Neue unwenig bekannte Taxa, neue Kombination un Synonyme Sowie Bermerkungen zu einigen Gattungen. J. Cramer, Berlin.

KRISTIANSEN, J. 1975. On the occurrence of the species of *Synura* (Chrysophyceae). Int. Ver. Theor. Angew. Limnol. Verh. 19: 2709–2715.

KRISTIANSEN, J. 1985. Occurrence of scale-bearing Chrysophyceae in a eutrophic Danish lake. Int. Ver. Theor. Angew. Limnol. Verh. 22: 2826–2829.

KRISTIANSEN, J. 1988. Seasonal occurrence of silica-scaled chrysophytes under eutrophic conditions. Hydrobiologia, 161: 171–184.

LEAVITT, P.R., AND D.L. FINDLAY. 1994. Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci. 51: 2286–2299.

LEAVITT, P.R., B.J. HANN, J.P. SMOL, B.A. ZEEB, C.E. CHRISTIE, B. WOLFE, AND H.J. KLING. 1994. Analysis of whole-lake experiments: an overview of results from Experimental Lakes Area Lake 227. Can. J. Fish. Aquat. Sci. 51: 2322–2332.

LEAVITT, P.R., S. CARPENTER, AND J.F. KITCHELL. 1989. Whole-lake experiments: the annual record of fossil pigments and zooplankton. Limnol. Oceanogr. 34: 700–717.

LEGENDRE, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology, 73: 1659–1673.

LEVINE, S.N., M.P. STAINTON, AND D.W. SCHINDLER. 1986. A radiotracer study of phosphorus cycling in a eutrophic Canadian Shield lake. Lake 227, northwestern Ontario. Can. J. Fish. Aquat. Sci. 43: 366–378.

LOTTER, A.F., AND H.J.B. BIRKS. 1993. The impact of the Laacher See Tephra on terrestrial and aquatic ecosystems in the Black Forest (Southern Germany). J. Quat. Sci. 8: 263–276.

MALLEY, D.F., P.S.S. CHANG, D.L. FINDLAY, AND G.A. LINSEY. 1988. Extreme perturbation of the zooplankton community of a small Precambrian Shield lake by the addition of nutrients. Int. Ver. Theor. Angew. Limnol. Verh. 23: 2237–2247.

NAUWERCK, A. 1963. Die Beziehungen zwischen Zooplankton und Phytoplankton im See Erken. Symb. Bot. Ups. 17(5): 163 p.

NICHOLLS, K.H. 1981. *Chrysococcus furcatus* (Dolg.) comb. nov.: a new name for *Chrysastrella furcata* (Dolg.) Defl. based on the discovery of the vegetative stage. Phycologia 20: 16–21.

NICHOLLS, K.H., AND E.C. CARNEY. 1979. The rare planktonic diatom *Eunotia zasuminensis* in Canada. Can. J. Bot. 57: 1150–1154.

NYGAARD, G. 1956. Ancient and recent flora of diatoms and Chrysophyceae in Lake Gribsø. Folia Limnol. Scand. 8: 32–262.

PATALAS, K. 1971. Crustacean plankton communities in 45 lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 231–244.

PATRICK R., AND C.W. REIMER. 1966. The diatoms of the United States exclusive of Alaska and Hawaii. Vol. 1. Livingston Publishing Co., Philadelphia, Penn.

PATRICK R., AND C.W. REIMER. 1975. The diatoms of the United States exclusive of Alaska and Hawaii. Vol. 2. Livingston Publishing Co., Philadelphia, Penn.

PREISIG, H.R., AND D.J. HIBBERD. 1982. Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera: 2. Nord. J. Bot. 2: 601–638.

PREISIG, H.R., AND D.J. HIBBERD. 1983. Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera: 3. Nord. J. Bot. 3: 695–723.

PRENTICE, I.C. 1980. Multidimensional scaling as a research tool in Quaternary palynology: a review of theory and methods. Rev. Palaeobot. Palynol. 31: 71–104.

RENBERG, I., AND H. HULTBERG. 1992. A paleolimnological assessment of acidification and liming effects on diatom assemblages in a Swedish lake. Can. J. Fish. Aquat. Sci. 49: 65–72.

ROIJACKERS, R.M.M., AND H. KESSELS. 1981. Chrysophyceae from freshwater localities near Nijmegen, The Netherlands. II. Hydrobiologia, 80: 231–239.

ROIJACKERS, R.M.M., AND H. KESSELS. 1986. Ecological characteristics of scale-bearing Chrysophyceae from the Netherlands. Nord. J. Bot. 6: 373–385.

RYBAK, M. 1986. The chrysophycean paleocyst flora of the bottom sediments of Kortowskie Lake (Poland) and its ecological significance. Hydrobiologia, 140: 67–84.

RYBAK, M. 1987. Fossil chrysophycean cyst flora of Racze Lake, Wolin Island (Poland) in relation to paleoenvironmental conditions. Hydrobiologia, 150: 257–272.

RYBAK, M., I. RYBAK, AND M. DICKMAN. 1987. Fossil chrysophycean cyst flora in a small meromictic lake in southern Ontario, and its paleoecological interpretation. Can. J. Bot. 65: 2425–2440.

RYBAK, M., I. RYBAK, AND K. NICHOLLS. 1991. Sedimentary chrysophycean cyst assemblages as paleoindicators in acid sensitive lakes. J. Paleolimnol. 5: 19–72.

SANDGREN, C.D. 1980a. An ultrastructural investigation of resting cyst formation in *Dinobryon cylindricum* Imhof (Chrysophyceae, Chrysophycota). Protistologica, 16: 259–275.

SANDGREN, C.D. 1980b. Resting cyst formation in selected chrysophyte flagellates: an ultrastructural survey including a proposal for the phylogenetic significance of interspecific variations in the encystment process. Protistologica, 16: 289–303.

SANDGREN, C.D. 1983. Survival strategies of chrysophycean flagellates: reproduction and the formation of resistant resting cysts, p. 23–48. *In* G.A. Fryxell [ed.] Survival strategies of the algae. Cambridge University Press, Cambridge, Mass.

SANDGREN, C.D. 1989. SEM investigations of statospore (stomatocyst) development in diverse members of the Chrysophyceae and Synuriphyceae. Beih. Nova Hedwigia, 95: 45–69.

SANDGREN, C.D., AND H.J. CARNEY. 1983. A flora of fossil Chrysophycean cysts from the recent sediments of Frains Lake, Michigan, USA. Nova Hedwigia, 38: 129–163.

SCHINDLER, D.W. 1977. Evolution of phosphorus limitation in lakes. Science (Wash., D.C.), 195: 260–262.

SCHINDLER, D.W., AND E.J. FEE. 1974. The Experimental Lakes Area: whole-lake experiments in eutrophication. J. Fish. Res. Board Can. 31: 937–953.

SCHINDLER, D.W., F.A.J. ARMSTRONG, S.K. HOLMGREN, AND G.J. BRUNSKILL. 1971. Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario by addition of phosphate and nitrate. J. Fish. Res. Board Can. 28: 1763–1782.

SCHINDLER, D.W., AND S.K. HOLMGREN. 1971. Primary production and phytoplankton in the Experimental Lakes Area (ELA) northwestern Ontario and other low-carbonate waters and a liquid scintillation method for determining ^{14}C activity in photosynthesis. J. Fish. Res. Board Can. 28: 189–201.

SCHINDLER, D.W., R.H. HESSLEIN, AND M.A. TURNER. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 6–25.

SCHINDLER, D.W., R.W. NEWBURY, K.G. BEATY, J. PROKOPOWICH, T. RUSZCZYNSKI, AND J.A. DALTON. 1980. Effects of a windstorm and forest fire on chemical losses from forested watersheds and on the quality of receiving streams. Can. J. Fish. Aquat. Sci. 37: 328–334.

SHEATH, R.G., J.A. HELLEBUST, AND T. SAWA. 1975. The statospore of *Dinobryon divergens* Imhof: formation and germination in a subarctic lake. J. Phycol. 11: 131–138.

SIVER, P.A. 1988. The distribution and ecology of *Spiniferomonas* (Chrysophyceae) in Connecticut (USA). Nord. J. Bot. 8: 205–212.

SIVER, P.A. 1991a. Implications for improving paleolimnological inference models utilizing scale-bearing siliceous algae: transforming scale counts to cell counts. J. Paleolimnol. 5: 219–225.

SIVER, P.A. 1991b. The biology of *Mallomonas*: morphology, taxonomy and ecology. Kluwer Academic Publishers, Boston.

SIVER, P.A., J.S. HAMER, AND H. KLING. 1990. The separation of *Mallomonas duerrschmidtiae* sp. nov. from *M. crassisquama* and *M. pseudocoronata*: implications for paleolimnological research. J. Phycol. 26: 728–740.

SKOGSTAD, A. 1986. *Chromophysomonas* (Chrysophyceae) from twenty-seven localities in the Oslo area, p. 259–269. *In* J. Kristiansen and R.A. Andersen [ed.] Chrysophytes: aspects and problems. Cambridge University Press, Cambridge, Mass.

SMOL, J.P. 1983. Paleophycology of a high arctic lake near Cape Herschel, Ellesmere Island. Can. J. Bot. 61: 2195–2204.

SMOL, J.P. 1995. Application of chrysophytes to problems in paleoecology. *In* C. Sandgren, J.P. Smol, and J. Kristiansen [ed.] Chrysophyte algae: ecology, phylogeny and development. Cambridge University Press, Cambridge, Mass. pp. 303–329.

SMOL, J.P., AND M.D. DICKMAN. 1981. The recent histories of three Canadian Shield lakes: a paleolimnological experiment. Arch. Hydrobiol. 93: 83–108.

SMOL, J.P., S.R. BROWN, AND R.N. MCNEELY. 1983. Cultural disturbances and trophic history of a small meromictic lake from central Canada. Hydrobiologia, 103: 125–130.

STOCKNER, J.G. 1971. Preliminary characterization of lakes in the Experimental Lakes Area, northwestern Ontario, using diatom occurrences in sediments. J. Fish. Res. Board Can. 28: 265–275.

STOCKNER, J.G., AND W.W. BENSON. 1967. The succession of diatom assemblages in the recent sediments of Lake Washington. Limnol. Oceanogr. 5: 195–211.

STOERMER, E.F., J.A. WOLIN, C.L. SCHELSKE, AND D.J. CONLEY. 1985. An assessment of ecological changes during the recent history of Lake Ontario based on siliceous algal microfossils preserved in the sediments. J. Phycol. 21: 257–276.

TER BRAAK, C.J.F. 1987–1990. CANOCO-a FORTRAN program for CANOnical Community Ordination. Microcomputer Power, Ithaca, N.Y.

WOLFE, B., H.J. KLING, G.J. BRUNSKILL, AND P. WILKINSON. 1994. Multiple dating of a freeze core from Lake 227, an experimentally fertilized lake with varved sediments. Can. J. Fish. Aquat. Sci. 51: 2274–2285.

YUNG, Y.-K., K.H. NICHOLLS, AND A.G. CHENG. 1988. The detection of *Rhizosolenia* (Bacillariophyceae) in sediment of Ontario lakes and implications for paleoecology. J. Paleolimnol. 1: 61–69.

ZEEB, B.A., AND J.P. SMOL. 1993. Chrysophycean stomatocyst flora of Elk Lake, Clearwater County, Minnesota. Can. J. Bot. 71: 737–756.

ZEEB, B.A., K.E. DUFF, AND J.P. SMOL. 1990. Morphological descriptions and stratigraphic profiles of chrysophycean stomatocysts from the recent sediments of Little Round Lake, Ontario. Nova Hedwigia, 51: 361–380.

Cladocera Community Response to Experimental Eutrophication in Lake 227 as Recorded in Laminated Sediments[1]

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The response of pelagic zooplankton to experimental fertilization was compared with the fossil record of Cladocera obtained from the annually laminated sediments of Lake 227, Experimental Lakes Area, northwestern Ontario. Constrained cluster analysis of both total fossil Cladocera and littoral chydorid communities clearly distinguished between pre- and post-eutrophication communities and further differentiated between years of high and low nitrogen:phosphorus fertilization ratios. However, there were few chydorid species extirpations resulting from the manipulation. Total chydorid accumulation rates and indices of species diversity, richness, and equitability were relatively constant over the last century and were not affected by fertilization. Among pelagic Cladocera, *Bosmina longirostris* abundance declined >60% after initial fertilization. Although harsh chemical conditions (pH >10) may have contributed to reduced abundance of pelagic Cladocera, *Bosmina* populations were also naturally variable prior to manipulation. Changes in *Bosmina* morphology (mucrone, antennule, and carapace length) and cladoceran size ratios (*Daphnia*/[*Daphnia* + *Bosmina*]) suggested that zooplanktivory by fish and invertebrates exercised important control of herbivore populations. Fossil *Bosmina* concentration (remains·[g dry wt]$^{-1}$ or remains·[g organic matter]$^{-1}$) were significantly correlated ($r = 0.66$, $P < 0.01$, 1970–1989) to standing crop in the water column (animals·m^{-2}). Fossil accumulation rate (remains·cm^{-2}·yr^{-1}) was not significantly correlated to *Bosmina* abundance, perhaps because of errors in determining bulk sediment accumulation rates.

On a comparé la réponse à une fertilisation expérimentale du zooplancton pélagique aux caractéristiques de cladocères fossiles contenus dans les sédiments formés en varves du lac 227, région des lacs expérimentaux. L'analyse par grappes regroupées du nombre des cladocères fossiles totaux ainsi que des communautés de chydoridés littoraux a permis de faire la distinction nette entre les communautés qui ont précédé et celles qui ont suivi l'eutrophisation, et elle a permis en outre de faire la différence entre les années de fertilisation que le rapport azote/phosphore était élevé ou bas. Cependant, la transformation du lac n'a pas fait disparaître beaucoup d'espèces de chydoridés. Les taux d'accumulation totale des chydoridés et les indices de diversité, de richesse et de la diversité observée à comparer au maximum possible sont restés assez constants au cours du dernier siècle et n'ont pas été perturbés par la fertilisation. Au sein du groupe des cladocères pélagiques, l'abondance de *Bosmina longirostris* a chuté de plus de 60 % après la fertilisation initiale. Même si les conditions ont pu être dures sur le plan chimique (pH > 10) et qu'elles ont pu contribuer à une réduction en abondance de ce cladocère pélagique, il demeure que les populations de *Bosmina* variaient déjà naturellement avant la transformation du lac. Des changements observés au niveau de la morphologie de *Bosmina* (mucrone, antennule et longueur de la carapace) ainsi que dans les rapports de taille entre cladocères (*Daphnia*/[*Daphnia* + *Bosmina*]) indiquent que la zooplanctophagie exercée par les poissons et d'autres invertébrés a exercé un important contrôle sur les populations d'herbivores. La concentration des *Bosmina* fossiles est en corrélation ($r = 0{,}66$, $P < 0{,}01$, 1970–1989) avec la biomasse de la colonne d'eau. Il n'y a pas de relation significative entre la vitesse d'accumulation des fossiles et l'abondance de *Bosmina*; cela peut être attribuable à des erreurs dans la détermination du taux d'accumulation général des sédiments.

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Experimental fertilization of Lake 227 began in 1969 and was the longest running manipulation at the Experimental Lakes Area (ELA), northwestern Ontario (Schindler 1987). Most pelagic Cladocera (*Bosmina longirostris*,

Diaphanosoma birgei, *Holopedium gibberum*), calanoid copepods (*Diaptomus minutus*), and rotifers declined to low levels after 3–5 yr of fertilization, perhaps as a result of toxic pH (>10) caused by excess primary production (Malley and Chang 1980; Malley et al. 1988a, 1988b). Despite well-documented changes in zooplankton after fertilization, little is known of the premanipulation communities or their baseline variability. Without such information, it is difficult to determine whether zooplankton community change was caused by fertilization or was a result of natural variability. Analysis of fossil Cladocera may be used to evaluate the historical significance of experimental eutrophication. This approach is particularly robust for Lake 227 because it has continuously laminated sediments which provide an accurate sediment chronology (Wolfe et al. 1994).

Cladocera are well preserved in lake sediments, in contrast to Copepoda and Rotifera, which are only represented by occasional reproductive structures (Frey 1988; Kitchell and Sanford 1992). Most parts of the exoskeletons from Bosminidae and Chydoridae are preserved. However, other cladocerans are represented variously by postabdomens, claws, mandibles, ephippia, and antennal segments, but not headshields or shells (Frey 1988; Hann 1989). Analysis of fossil cladoceran communities has been useful in identifying eutrophication events (Boucherle and Züllig 1983; Whiteside and Swindoll 1988), as well as changes in predation by fish (Hrbáček 1969; Kerfoot 1974; Kitchell and Carpenter 1987; Leavitt et al. 1989) and invertebrates (Kerfoot 1981; Brugam and Speziale 1983; Salo et al. 1989). Despite widespread use of Cladocera in paleolimnology (Hofmann 1986; Frey 1988; Hann 1989), few investigators have quantified the relation between secondary production and fossil deposition (Deevey 1964; Deevey et al. 1977; Kerfoot 1974).

This study had two goals: (1) to assess the effects of whole-lake eutrophication from an analysis of pre- and post-manipulation fossil Cladocera; and (2) to compare the fossil record with 20 yr of contemporaneous zooplankton collections. As part of our first objective, we also sought to use changes in *Bosmina* morphology to infer how zooplanktivory by fish and invertebrate predators (*Chaoborus*) regulates herbivore populations (Kerfoot 1981, 1987). Neither predator is well documented in historical records from the ELA, and their effects can only be reconstructed from the fossil record.

Materials and Methods

Site Description

Lake 227 is a small (5 ha), relatively deep (10 m), headwater lake located on Canadian Shield bedrock in the ELA (49°42′N, 93°42′W). Regional characteristics and basin morphometry are described elsewhere (Brunskill and Schindler 1971; Cleugh and Hauser 1971). Details of experimental eutrophication are presented by Schindler (1977, 1987). Fertilization between 1969 and 1974 increased inputs of nitrogen (N) and phosphorus (P) (13:1 mass ratio) 10-fold over natural fluxes. After 1975, N inputs were reduced to a 5:1 ratio with P. Nitrogen fertilization was discontinued after 1989.

Planktivorous cyprinids dominate the fish community; these include fathead minnow (*Pimephales promelas*), pearl dace (*Margariscus margarita*), northern redbelly dace (*Phoxinus eos*), and finescale dace (*Phoxinus neogaeus*) (Beamish et al. 1976). Predaceous invertebrates include cyclopoid copepods (*Tropocyclops prasinus mexicanus*, *Mesocyclops edax*, *Cyclops bicuspidatus thomasi*) and dipteran larvae (*Chaoborus flavicans*, *C. punctipennis*) (Malley et al. 1988a). Current pelagic zooplankton are small-bodied and dominated by *Bosmina longirostris* and *Diaptomus minutus* (P.S.S. Chang, unpublished data).

Plankton Methods

Zooplankton were sampled either weekly (1969–1971), biweekly (1972–1975, 1977, 1979–1982, 1988–1990), or monthly (1976, 1978, 1983–1987) during the ice-free season. Samples were collected at midday from a central station using either a transparent Schindler-Patalas trap (1969–1977), a four-barrel closing sampler (1978–1982), or a two-barrel sampler (after 1983) (Chang et al. 1980, 1981; Malley et al. 1988a, 1988b). Zooplankton were collected separately from the epi-, meta-, and hypolimnion, except from 1975 to 1976 when only the upper 2 m were sampled. *Chaoborus* collections were incidental in daytime zooplankton samples until 1986 when monthly nighttime collections were initiated. Littoral microcrustaceans were never quantitatively sampled. Zooplankton standing crops were calculated as volume-weighted, monthly average density (animals·m^{-2}).

Sediment Analyses

Sediments were collected from the deep portion of the basin using a freeze corer during January 1990 (Leavitt and Findlay 1994). Approximately annual increments were isolated from freeze-dried sediments (based on color, texture, laminae, and sediment coherence) and placed in individual vials (Leavitt and Findlay 1994). Samples were further freeze-dried for 2 h before subsampling. Tared samples were soaked overnight in 10% KOH at room temperature and gently swirled before concentration of microfossils on a 53 μm screen (Hann 1989). Slides for enumeration were prepared by dispersing 50 μL of an aqueous suspension of microfossils into glycerine jelly and covering with a coverslip. Cladoceran remains were counted until at least 150 chydorids or, rarely, 10 slides were enumerated.

Cladoceran microfossils were identified to species and verified using reference slides from the region (Hann 1981). Remains were tabulated as head shields, carapaces or "others" (mandibles, postabdomens, claws, antennal segments), and the most numerous remains were used to calculate microfossil abundance. Annual fossil deposition was expressed as accumulation rate (remains·cm^{-2}), concentration per unit organic matter (remains·[g organic matter]$^{-1}$), or concentration per unit sediment weight (remains·[g dry wt]$^{-1}$). Organic content was estimated by weight loss on ignition for 1 h at 500°C (Dean 1974). Relative cladoceran abundance was calculated as a percentage of the fossil sum. Taxa that occurred in only one sample, or were always <5% of the fossil sum, were recorded as either present or absent.

Changes in the predation regime of Lake 227 were inferred from an analysis of *Bosmina longirostris* morphology (O'Brien and Schmidt 1979; Sanford 1993). Size-selective predation by zooplanktivorous fish reduces mean *Bosmina* body length (Kerfoot 1981; Salo et al. 1989). Similarly, *Bosmina* with long antennules or mucrones are less sensitive to predation by invertebrates than short-featured individuals (Kerfoot 1974, 1981, 1987; Sprules et al. 1984). Carapace,

TABLE 1. Species list of Cladocera remains recovered from the Lake 227 core.

Family Chydoridae
Eurycercus (*Bullatifrons*) *longirostris* Hann, 1982
Alonella excisa (Fischer, 1854)
A. exigua (Lilljeborg, 1853)
A. nana (Baird, 1850)
A. pulchella Herrick, 1884
Disparalona rostrata (Koch, 1841)
D. acutirostris (Birge, 1878)
Chydorus brevilabris Frey, 1980
C. piger Sars, 1862
C. faviformis Birge, 1893
Alona affinis (Leydig, 1860)
A. quadrangularis (O.F. Müller, 1785)
A. intermedia Sars, 1862
A. circumfimbriata Megard, 1967
A. setulosa Megard, 1967
A. barbulata Megard, 1967
A. costata Sars, 1862
A. rustica Scott, 1895
A. guttata Sars, 1862
Acroperus cf. *harpae* Baird, 1843
Camptocercus sp.
Graptoleberis testudinaria (Fischer, 1848)
Family Bosminidae
Bosmina longirostris (O.F. Müller, 1785)
Family Daphniidae
Daphnia sp.
Family Polyphemidae
Polyphemus pediculus (Linnaeus, 1761)
Family Leptodoridae
Leptodora kindti (Focke, 1844)
Family Macrothricidae
Ophryoxus gracilis Sars, 1861
Acantholeberis curvirostris (O.F. Müller, 1776)
Ilyocryptus sordidus (Lieven, 1848)
Family Sididae
Sida crystallina (O.F. Müller, 1785)
Latona setifera (O.F. Müller, 1785)

antennule, and mucrone lengths were measured according to Kerfoot (1975). An average of 20 (minimum of 10) measurements for each morphological feature were obtained at each stratigraphic level.

The size-structure of the pelagic cladoceran community was quantified as the ratio of fossil *Daphnia*/(*Daphnia* + *Bosmina*) (as % *Daphnia*; Leavitt et al. 1989). This ratio has been used as an indicator of zooplanktivory by fish in lakes where the zooplankton taxa coexist and where *Daphnia* are >1 mm in length (Carpenter et al. 1987; Carpenter 1989; Leavitt et al. 1989, 1993). High levels of vertebrate zooplanktivory are characterized by low abundance (<20%) of large *Daphnia* remains relative to the sum of *Daphnia* + *Bosmina*.

Total herbivory by zooplankton was estimated using fossil pheophorbide *a*, a chlorophyll derivative produced during passage through herbivore guts (Daley 1973; Carpenter and Bergquist 1985). Fossil concentrations of pheophorbide *a* were quantified by high pressure liquid chromatography as reported by Leavitt and Findlay (1994), and were expressed as nmol pheophorbide *a*·(g organic matter)$^{-1}$.

Statistical Analyses

Constrained cluster analysis of either total cladoceran or chydorid-only fossil concentrations was used to identify transitions between pre- and postmanipulation communities (Grimm 1987). This multivariate technique uses Ward's Minimum Variance method (incremental sum of squares) to calculate a dissimilarity matrix of Euclidean distances between adjacent samples based on percentage composition. At each stage of analysis, adjacent clusters are examined and pairs of clusters with the smallest dissimilarity value are merged (Grimm 1987).

The effects of fertilization were also identified by changes in the species diversity and equitability of fossil cladoceran communities. The Shannon–Wiener index of diversity (H', Pielou 1977) is based on the proportional abundance of species, and takes into account both species richness and evenness (Magurran 1988). Species equitability was calculated according to methods detailed in Lloyd and Ghelardi (1964), in which observed diversity is compared with the "ecological maximum" diversity for a given number of species.

Results

Cladocera in the Fossil Record

The remains of 22 species of chydorids were recovered from Lake 227 (Table 1) and included representatives of all major regional communities (Hann 1981). Sediments also contained taxa that are rare in northwestern Ontario (*Alonella pulchella*, *Chydorus faviformis*, *Alona intermedia*, *Alona barbulata*). Fossils of nine species of nonchydorid Cladocera were also typical of regional fauna (Patalas 1971); *Bosmina longirostris* was the dominant fossil taxon (Table 1). The genus *Daphnia* (*retrocurva*, according to Malley et al. 1988a) was represented by mandibles and a few postabdominal claws and trunk limbs. Occasional remains included large predaceous taxa (*Polyphemus pediculus*, *Leptodora kindti*) and several macrothricids and sidids. In contrast, microfossil remains of *Holopedium gibberum* and *Diaphanosoma birgei* were not recovered despite being present in the plankton in low abundance. Postabdominal claws are often the most common remains of these small pelagic Cladocera, and are not quantitatively retained on the 53 μm screen.

Response of Cladocera to Eutrophication

Constrained cluster analysis of cladoceran remains identified two major stratigraphic zones that correspond to pre- and postmanipulation communities (Fig. 1). A further three clusters were differentiated in recent sediments corresponding to years of high N:P ratio (1972–1975), low N:P ratio (1976–1982), and low N input from 1982 onwards. Separate analysis of chydorid assemblages (not shown) revealed similar clusters, although high and low N:P years were less clearly demarcated. In both cluster analyses, samples from 1969–1971 were grouped with the premanipulation cluster, suggesting a lag in community response to fertilization.

Chydorids

Populations of several littoral Chydoridae changed in response to fertilization of Lake 227 (Fig. 1). *Chydorus brevilabris*, a rare taxon before 1969, displayed a marked increase in abundance and more consistent occurrence coincident with nutrient addition. *Alona quadrangularis* was most common during years of high N fertilization (1969–1974). Several

other species of *Alona* (*A. circumfimbriata*, *A. setulosa*, and *A. guttata*) were most abundant in the last 15 yr. In contrast, *Disparalona acutirostris*, *Alona intermedia*, and *Alonella excisa* were all less abundant after 1975 than in the 20 yr before eutrophication.

Fossil chydorid species diversity, richness, and equitability did not change greatly as a result of whole-lake eutrophication (Fig. 2). Species richness and diversity were strongly correlated ($r = 0.934$, $P = 0.0001$). Chydorid diversity was greatest during the early 1900s (not shown) and at the start of fertilization, whereas communities were depauperate during three periods: 1961–1963, 1973–1975, and the mid-1980s. Equitability (mean $\epsilon \pm$ SE = 1.17 ± 0.03) fell below 1.0 on three occasions only. Although all indices declined markedly in the early 1970s, these changes were similar to those noted before manipulation (1955–1965) and during the 1980s, when nutrient inputs were constant (Fig. 2).

The total chydorid accumulation rate varied throughout the latter half of the 20th century and did not show a strong response to eutrophication (Fig. 3a). Deposition of chydorids peaked at the onset of fertilization (1968–1970), but similar maxima were observed during periods of constant nutrient flux, both before (1952–1958) and after manipulation (1980–1982, 1985). In contrast, chydorid abundance appeared to decline after fertilization when fossil concentrations were expressed as remains·(g organic matter)$^{-1}$ (Fig. 3b) or remains·(g dry weight)$^{-1}$ (not shown).

Non-chydorid Cladocera

Accumulation rates of the main pelagic cladoceran, *Bosmina longirostris*, appeared to decline after fertilization (Fig. 3a). Declines in *Bosmina* abundance since 1950 were further accentuated when fossil concentrations were calculated per unit organic matter (Fig. 3b). The loss of *Bosmina* was particularly marked when comparing concentrations from the two periods of constant nutrient flux (i.e., 1975–1989 and premanipulation). The reduction in organic-specific *Bosmina* concentration was 62.7% when compared with mean herbivore abundance from 1898 to 1968, and far exceeded the relative increase in percent organic matter (26.4%) over the same periods. Thus, observed declines in *Bosmina* were not the sole result of fossil dilution by increased primary production.

Although *Bosmina longirostris* was the dominant pelagic fossil prior to manipulation, remains from *Daphnia* spp. were consistently present at 10–20% relative abundance (Fig. 4). *Daphnia* increased to 45% relative abundance after nutrient addition, but populations were characterized by high variability, with maxima in 1973–1975, 1981–1983, and 1989 (Fig. 4). These peaks resulted from both reduced *Bosmina* deposition and increased *Daphnia* abundance (Fig. 3). Although small *Daphnia retrocurva* predominated in the epilimnetic waters of Lake 227, historical records indicated that metalimnetic *Daphnia* spp. exceeded 1 mm length during the mid-1970s (P.S.S. Chang, unpublished data). Early peaks of *Daphnia* also corresponded to years in which *Chaoborus punctipennis* and *C. flavicans* were observed in elevated numbers in incidental collections (Malley et al. 1988b; D. Malley, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data).

Community Grazing

Concentrations of fossil pheophorbide in Lake 227 sediments increased four-fold during the early 1970s when

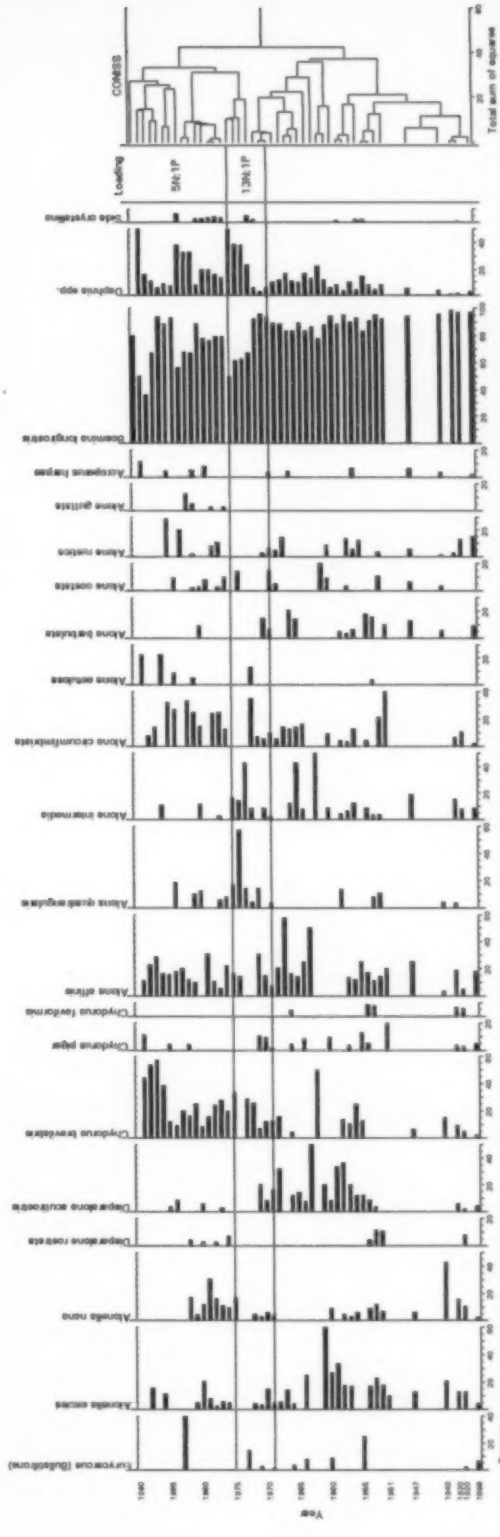

FIG. 1. Relative abundance (% of chydorid sum and nonchydorid sum, respectively) of chydorids and other Cladocera in sediments from Lake 227. Constrained cluster analysis (CONISS, Grimm 1987) is presented at right.

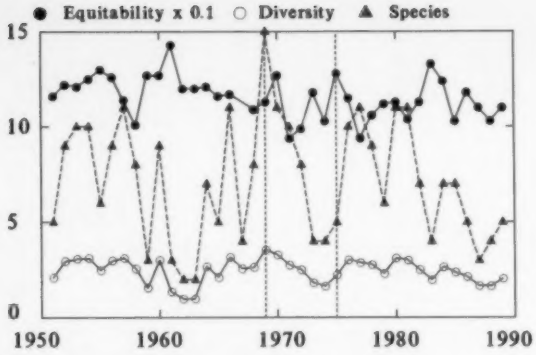

FIG. 2. Fossil chydorid species richness, diversity, and equitability in Lake 227, 1940–1990. Vertical dotted lines denote the period of fertilization using a nitrogen:phosphorus ratio of 13:1 (by mass; see Fig. 1).

N:P ratios were high, but declined to twice premanipulation values after 1975 (Fig. 4). Fossil pheophorbide *a* concentrations were also strongly correlated ($r = 0.64$, $P < 0.001$) to percent *Daphnia* between 1940 and 1989, but not with absolute fossil *Daphnia* abundance (remains·[g organic matter]$^{-1}$ or remains·cm^{-2}·yr^{-1}).

Bosmina Morphology

Fossil *Bosmina* populations were composed of remains from small-bodied individuals (Fig. 5a; cf. Kerfoot 1981). *Bosmina* were significantly larger during years of high, constant nutrient input (mean carapace length + SE = 221 ± 5 μm; 1975–1990) than during the premanipulation period (204 ± 4 μm; 1898–1968). Body size was smallest after initial fertilization (1973–1974), although small *Bosmina* were also common in 1961 and 1968.

Fossil bosminids were characterized by long mucrones (Fig. 5b). Mucrone length averaged 25% of carapace length and was relatively constant through the premanipulation era. Mucrone length increased during the early 1970s and was generally high, although variable, for the rest of the decade. Beginning in 1981, mucrone length declined continuously to an historical minimum in the most recent sediments.

Fossil *Bosmina* populations were composed almost exclusively of a morphotype with strongly recurved antennules, *B. longirostris* f. *cornuta* (Sanford 1993; Fig. 5c). *Bosmina* with straight antennules made up 10–20% of the population through much of the 1950s and 1960s, but were absent in the mid-1970s and throughout the 1980s. This recent loss of straight-form antennules was also reflected in striking reductions in mean antennule length during the 1980s, after historical maxima during the late 1970s (Fig. 5c).

Comparison of Plankton and Fossil Records

Among pelagic taxa, only *Bosmina longirostris* was sufficiently common in both plankton and fossil data sets to allow direct comparison. Plankton–fossil comparisons for other taxa, including *Daphnia* spp., were hampered by incomplete water column records (ice-free season only), and changes in zooplankton sampling methodology. Water column samples were dominated by copepods and rotifers (Malley et al. 1988a), which are poorly preserved in lake sediments

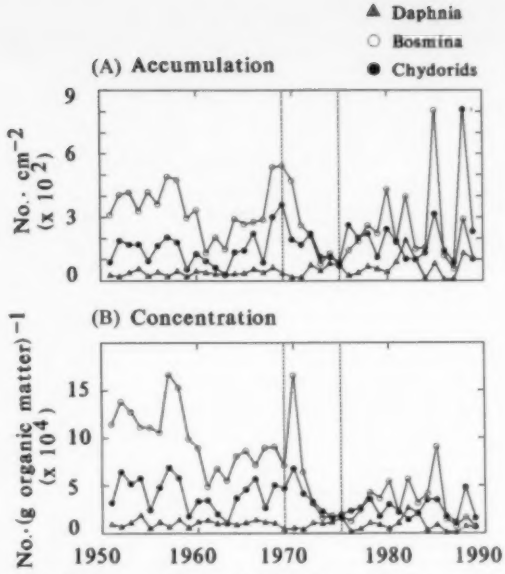

FIG. 3. Abundance of fossil Chydoridae (solid circle), *Bosmina longirostris* (open circle,) and *Daphnia* spp. (hatched triangle) calculated as: (A) accumulation rate (remains·cm^{-2}) and (B) organic matter-specific concentration (remains·[g organic matter]$^{-1}$). Vertical dotted lines as in Fig. 2.

(Frey 1988). In contrast, sediment samples contained abundant remains from littoral communities that were not sampled during the manipulation.

Annual ice-free standing stocks of *Bosmina longirostris* were significantly correlated to fossil concentrations expressed per g organic matter ($r = 0.66$, $P = 0.002$) or per gram dry weight ($r = 0.60$, $P = 0.007$; Fig. 6). The correspondence was particularly strong between 1970 and 1975 when plankton sampling was most intense (Malley et al. 1988a), and somewhat weaker after 1975 when sampling frequency became more variable. In contrast, the fossil accumulation rate (remains·cm^{-2}·yr^{-1}) was poorly correlated to *Bosmina* standing crop ($r = 0.21$, $P > 0.3$).

Discussion

Comparison of fossil and coeval historical records allowed us to determine the reliability of the paleoecological approach. As in earlier studies (reviewed in Frey 1988), we found that only selected taxa were represented in the fossil zooplankton community. Microfossils of rotifers and copepods were rare or absent, as were those from large, infrequently occurring taxa such as *Chaoborus*, which often require large samples in order to be accurately quantified. Among the Cladocera, those taxa with well-sclerotized exoskeletons, especially the chydorids and bosminids, were well represented. In contrast, cladocerans with thinner exoskeletons (e.g., daphnids, macrothricids, sidids) were rare and had to be quantified from mandibles, claws, or antennal segments. Despite selective preservation, fossil analyses provided critical information that was not available from historical records and which could be used as baseline data for evaluating the impact of eutrophication.

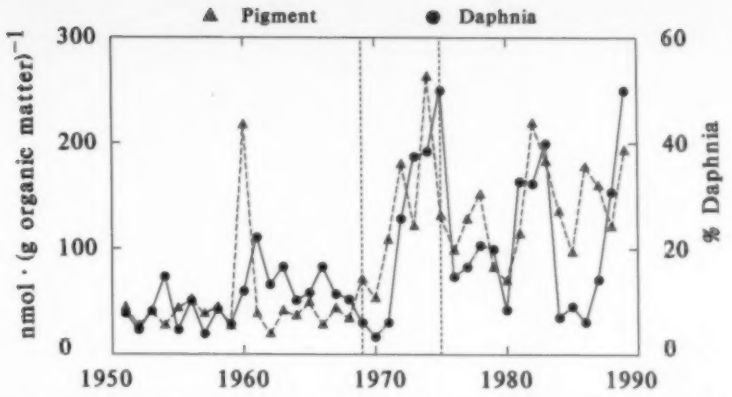

FIG. 4. Percent abundance of *Daphnia* remains relative to *Bosmina* + *Daphnia*, and concentration of pheophorbide *a* (nmol pigment·[g organic matter]$^{-1}$) in sediments. Vertical dotted lines as in Fig. 2.

Premanipulation Cladoceran Communities

Cladoceran communities were unexpectedly variable prior to the onset of fertilization (Fig. 1). Accumulation rates and fossil concentrations of total chydorids and *Bosmina* declined sharply between 1955 and 1963, similar to reductions during fertilization (Fig. 3). Acidophilic chydorids (*Disparalona acutirostris*, *Alona intermedia*, *Alonella excisa*; B.J. Hann, unpublished data) all reached historical maxima during the late 1950s and early 1960s, particularly at the expense of *Alonella nana*. *Daphnia* abundance also increased relative to *Bosmina* during the 1960s (Fig. 4). In contrast, chydorid diversity, species richness (Fig. 2), and total abundance (Fig. 3) all declined to historical minima during the early 1960s. Similar premanipulation changes are known from analyses of algal microfossils (Zeeb et al. 1994). Such background variability is surprising in view of the recent stability of reference lakes in the ELA (Schindler et al. 1990), and the undisturbed, headwater nature of Lake 227.

Some background variability may have been due to changes in predation by fish and invertebrates. Prior to fertilization, Lake 227 was naturally colored (18–25 Pt units, Brunskill et al. 1971), strongly stratified (Schindler et al. 1971), and dominated by small cyprinids (Beamish et al. 1976). This combination of traits is common in lakes subject to periodic winterkill. Coincidental increases in mean *Bosmina* body size and a shift towards larger *Daphnia* during the 1960s are consistent with a partial elimination of zooplanktivorous fish by winterkill (Leavitt et al. 1989). However, it is presently unknown how winterkill might affect chydorid communities.

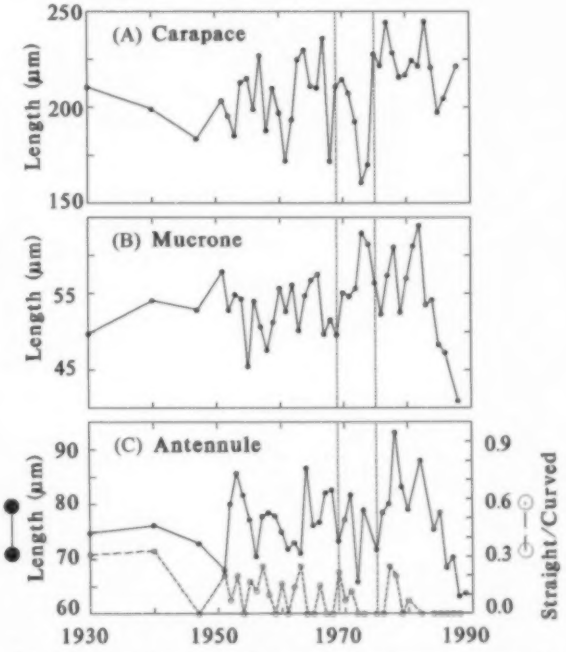

FIG. 5. Morphology of fossil *Bosmina longirostris* in Lake 227: (A) carapace length, (B) mucrone length, and (C) antennule length (solid circles) and relative abundance of straight-antennule morphs (open circles). Vertical dotted lines as in Fig. 2.

Effects of Eutrophication on Cladocera

Constrained cluster analysis delimited postfertilization cladoceran communities (both total Cladocera and Chydoridae alone) and premanipulation assemblages, despite high natural variability in cladoceran populations. Cluster analysis also separated the postfertilization communities into three distinct assemblages corresponding to years of high N:P ratio, low N:P ratio, and recent communities (after 1982). Despite clear delimitation of these communities, changes in species composition were generally subtle, and clusters were usually defined by changes in the relative abundance of species rather than by species replacements. This suggests that nutrient flux or associated features (e.g., light penetration, pH, algal community) were important determinants of cladoceran community structure, and that each nutrient regime (high N:P, low N:P, no fertilization) had a characteristic assemblage composition.

Cluster analysis also indicated that the full effects of fertilization on cladoceran communities took several years to become evident. Fossil samples from 1969 to 1972 were more similar to those from 1960 to 1968 than to those from the mid-1970s (Fig. 1). Similarly, chydorid diversity declined steadily after initial fertilization and reached a minimum in 1974 (Fig. 2). These fossil patterns mirror contemporaneous zooplankton records (Malley et al. 1988b), which show that

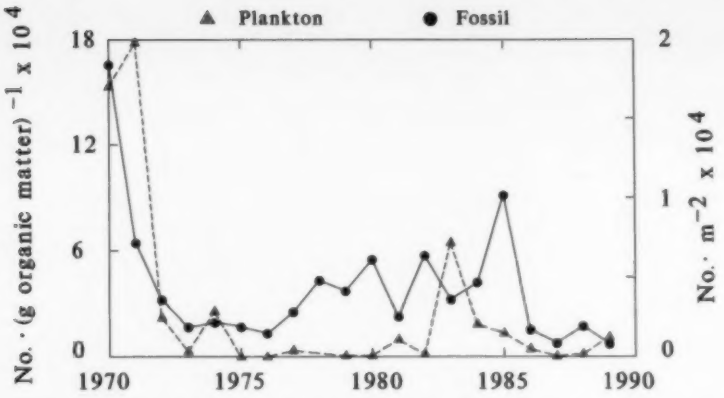

FIG. 6. Abundance of fossil *Bosmina* (remains·[g organic matter]$^{-1}$) and planktonic *Bosmina* (animals·m^{-2}) in Lake 227. See Methods for changes in plankton sampling protocol.

changes in crustacean diversity and zooplankton biomass were slight until 1972, after which declines were more marked.

Chydorids

Chydorid community composition was apparently impacted by a complex mixture of biotic and abiotic controls. For example, 10-fold increases in P inputs produced massive blooms of green algae and colonial cyanobacteria (Schindler et al. 1973; Leavitt and Findlay 1994). *Chydorus brevilabris* may have benefitted from these blooms by using floating algal mats to expand their habitat beyond the littoral zone. Such behavior has been reported for *C. sphaericus* (Frey 1988). In contrast, some taxa that declined during fertilization (*Disparalona acutirostris*, *Alona intermedia*, *Alonella excisa*) prefer acidic habitats (B.J. Hann, unpublished data) and may have been eliminated when epilimnetic waters exceeded a value of pH 10 (Malley et al. 1988a).

Some chydorid species may have been affected by changes in microhabitat structure. Generally, the species that prefer sediment microhabitats increased following manipulation of Lake 227 (e.g., *Alona quadrangularis* and *A. circumfimbriata*; Goulden 1971; Whiteside et al. 1978; Chengalath 1982, 1987). In contrast, *Chydorus faviformis* is strongly associated with vegetation (Whiteside et al. 1978) and was relatively rare after fertilization began. Surface blooms of cyanobacteria dominated in Lake 227 (Leavitt and Findlay 1994) and are known to shade-out macrophytes in other eutrophic lakes (Kitchell 1992). Overall, the replacement of phytophilic taxa by meiobenthic and facultatively planktonic species parallels chydorid community changes observed among lakes arranged along a gradient of increasing productivity (Whiteside 1970). Unfortunately, further detailed interpretation of species changes is difficult because little is known of the optima and preferences of many chydorids with respect to water chemistry and substrata (Frey 1988; Whiteside and Swindoll 1988).

Not all indices of chydorid community structure recorded a response to fertilization. Few species were permanently eliminated; consequently, community diversity, species richness, and equitability were relatively stable, especially considering the magnitude of the manipulation (Fig. 2). Fossil pigment analyses showed that experimental fertilization was the strongest eutrophication event in more than 100 yr (Leavitt and Findlay 1994). Whiteside and Harmsworth (1967) and Whiteside (1969) have demonstrated a strong negative correlation between chydorid diversity and primary productivity. Similarly, Whiteside and Swindoll (1988) predicted that an increase in nutrients should strongly depress chydorid diversity and favor planktonic Cladocera such as *Bosmina* and *Chydorus sphaericus*. Although chydorid diversity in Lake 227 did decline rapidly soon after fertilization began, observed changes were similar to those seen prior to manipulation (1961–1963) and may not be directly attributable to eutrophication (cf. Whiteside 1983). Instead, our results are similar to those of Hofmann (1986) who found that littoral chydorid community composition was stable through the Holocene, despite documented eutrophication. These results suggest that general indicators of chydorid community composition are relatively insensitive to community changes resulting from eutrophication.

Nonchydorids

Some pelagic Cladocera were selectively reduced as a result of fertilization. Both accumulation- and concentration-based estimates of *Bosmina* abundance declined by more than 60% within 4 yr of eutrophication (Fig. 3). In fact, historical records suggest that all planktonic crustaceans were rare during ice-free seasons between 1972 and the mid-1980s (Malley et al. 1988a; D. Malley, unpublished data). Malley et al. (1988b) have argued that pelagic crustaceans were eliminated by high pH and severe hypolimnetic oxygen deficits resulting from elevated primary production. Enclosure experiments have demonstrated that high pH can selectively eliminate planktonic Cladocera (Hansen et al. 1991), perhaps by inhibiting maintenance of sodium balance (Potts and Fryer 1979). *Bosmina* may have been more sensitive to changes in water chemistry than were some benthic chydorids because summer epilimnetic pH often exceeded 10, whereas waters at >3 m depth remained at the premanipulation pH of 6.5 (Schindler et al. 1973).

Unlike reports from historical data, fossil analyses suggested that not all pelagic zooplankton were harmed by eutrophication. Accumulation and concentration of *Daphnia* fossils actually increased slightly after fertilization (Fig. 3), as predicted by some food-web theory (McQueen et al. 1986). These large *Daphnia* are more effective herbivores than similar-sized copepods or smaller crustaceans (Carpenter et al. 1987; Carpenter 1989). Increased herbivory associated

with *Daphnia* was apparently shown by three- to four-fold increases in the concentration of the grazing indicator pheophorbide *a* after eutrophication (Fig. 4; Levitt and Findlay 1994). Pheophorbide *a* was also strongly correlated with the relative abundance of *Daphnia* in Lake 227 throughout the last 40 yr ($r = 0.64$, $P < 0.001$), as observed in other lakes with manipulated food webs (Carpenter et al. 1988; Leavitt et al. 1989, 1993). Large *Daphnia* may have been less affected than *Bosmina* by high epilimnetic pH because *Daphnia* also inhabited the metalimnion, a region with circumneutral pH (Schindler et al. 1973; P.S.S. Chang, unpublished data).

Effects of Vertebrate and Invertebrate Predators

Size-selective predation by fish and invertebrates may have been an important population regulator in Lake 227. *Bosmina* in the lake have always had extremely short antennules (mean length ± SE = 78 ± 0.8 μm), but relatively long mucrones (54 ± 0.5 μm), for such small carapaces (216 ± 7 μm) (Fig. 5; see Kerfoot 1981). This combination of traits suggests that both fish and copepods are important predators on *Bosmina* (Kerfoot 1974, 1981; Sprules et al. 1984; Whiteside and Swindoll 1988). Size-selective predation by fish reduces the upper limit of *Bosmina* body size, whereas copepod predation selects for resistant morphologies with long mucrones (Kerfoot 1974, 1981, 1987), but not necessarily long antennules (Sprules et al. 1984).

Sharp reductions in *Bosmina* body size between 1970 and 1973 suggest that predation by fish intensified early in eutrophication (Fig. 5a). Historical records support this interpretation and show that large pelagic Cladocera were rare during 1972–1973, whereas small taxa remained abundant. However, minnow populations were reduced by severe winterkill in the spring of 1974 (Malley et al. 1988a; K. Mills, Department of Fisheries and Oceans, Winnipeg, Man., personal communication). The reduction in predation by fish was apparently recorded by a return of fossil *Bosmina* to sizes characteristic of the premanipulation era (Fig. 5a), and by an increase in the relative abundance of fossil *Daphnia* (Fig. 4). Large-bodied *Daphnia* and *Chaoborus flavicans* were present in plankton tows during the summer of 1974 (Malley et al. 1988b; P.S.S. Chang, unpublished data). Large invertebrates are normally eliminated by cyprinids; therefore, their presence in Lake 227 is a sign of weak zooplanktivory by fish (Johnson et al. 1990; Uutala 1990; Leavitt et al. 1993; Carpenter and Kitchell 1993).

High planktivory by minnows between 1969 and 1973 may have also altered the relative importance of invertebrate predators. Increased predation by fish would be expected to selectively eliminate larger *Chaoborus flavicans* over smaller cyclopoid copepods. In addition, loss of *Chaoborus* should reduce mortality and increase survivorship of cyclopoid copepods, a preferred prey of *Chaoborus* (Neill and Peacock 1980; Elser et al. 1987; Yan et al. 1991). Fossil *Bosmina* showed increased mucrone length (Fig. 5b) between 1970 and 1974, consistent with elevated predation by copepods (Kerfoot 1981, 1987; Sprules et al. 1984).

Our results generally support the hypothesis that *Bosmina* morphology is a sensitive indicator of predation regimes in lakes, particularly those dominated by cyprinids (Kerfoot 1981; Whiteside and Swindoll 1988; Sanford 1993). However, some aspects of the fossil record remain difficult to interpret. For example, *Bosmina* antennule and mucrone length declined dramatically in the 1980s (Fig. 5), suggesting a sharp reduction in predation by invertebrates. At the same time, *Bosmina* body size was high relative to historical values, implying reduced planktivory by fish. Sustained low zooplanktivory could result from a series of severe winterkill events that eliminated both fish and invertebrates. Although high pH (>9) and low oxygen levels (<2 mg $O_2 \cdot L^{-1}$) co-occurred frequently in spring during the 1980s (B. Parker, Department of Zoology, University of Alberta, personal communication), fish and invertebrate survey data were insufficient to evaluate the importance of winterkill in structuring the food web of Lake 227.

Comparison of Plankton and Fossil Records of *Bosmina*

Direct comparison of *Bosmina* standing crop and fossil concentration showed that zooplankton abundance can be accurately reconstructed from annual fossil records. Correlations between *Bosmina* and fossil concentration were significant ($r > 0.6$, $P < 0.05$, 1970–1989) despite the lack of winter sampling, changes in field methodology (Fig. 6), high variability of field samples (Malley at al. 1988b), and the potentially confounding influence of sediment focusing (Anderson et al. 1987). Similar results were reported by Deevey (1964) and Leavitt et al. (1993) who found good correspondence between *Bosmina* abundance in the water column and those in trap and sediment samples. Deevey et al. (1977) also found good preservation of *Eubosmina tubicens* compared with *Cypria petenensis*, an ostracod that was selectively lost during deposition. Although Kerfoot (1981) estimated that more than 85% of production was lost during deposition, much of this loss may have been caused by degradiation of exuviae during sinking (Leavitt et al. 1993). Overall, these studies suggest that zooplankton can be deposited in direct proportion to their abundance in the water column, although there may be species- or lake-specific differences in the actual fraction of the standing crop that is deposited.

Our finding that fossil *Bosmina* concentration, but not the accumulation rate, was significantly correlated to zooplankton standing crop may also result from difficulty in accurately estimating bulk sediment accumulation rates (Leavitt and Findlay 1994). *Bosmina* accumulation was calculated as the product of fossil concentration (remains·[g dry wt]$^{-1}$) and bulk sediment accumulation rate ($g \cdot cm^{-2} \cdot yr^{-1}$). However, estimates of bulk accumulation may have been imprecise because of the difficulty in estimating the depositional area based on measurements of freeze-dried material. In contrast, estimates of fossil concentration required only accurate determination of sediment mass and weight loss on ignition, both of which are easily measured. Further research is required on the relative accuracy of concentration- and accumulation-based estimates of fossil abundance.

In summary, we found that *Bosmina* microfossils were deposited in proportion to standing crop and were reliable indicators of past population size. In general, analysis of cladoceran microfossils demonstrated that experimental fertilization altered the relative abundance of chydorid species because of a combination of changes in nutrient input, pH, and benthic microhabitat structure. Although declines in pelagic *Bosmina longirostris* may also have resulted from high pH values, changes in prey morphology and zooplankton size structure suggest that zooplanktivory also regulated herbivore populations. Unexpectedly, our study also showed

that similar changes in pelagic zooplankton occurred prior to fertilization, perhaps because of periodic winterkill events.

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References

ANDERSON, R.F., S.L. SCHIFF, AND R.H. HESSLEIN. 1987. Determining sediment accumulation and mixing rates using ^{210}Pb, ^{137}Cs, and other tracers: problems due to postdepositional mobility or coring artifacts. Can. J. Fish. Aquat. Sci. 44(Suppl.1): 231–250.

BEAMISH, R.J., L.M. BLOUW, AND G.A. MCFARLANE. 1976. A fish and chemical study of 109 lakes in the Experimental Lakes Area, northwestern Ontario, with appended reports on lake whitefish ageing errors and northwestern Ontario baitfish industry. Can. Fish. Mar. Serv. Res. Dev. Tech. Rep. 607.

BOUCHERLE, M.M., AND H. ZÜLLIG. 1983. Cladoceran remains as evidence of change in trophic state in three Swiss lakes. Hydrobiologia, 103: 141–146.

BRUGAM, R.B., AND B. SPEZIALE. 1983. Human disturbance and the paleolimnological record of change in the zooplankton community of Lake Harriet, Minnesota. Ecology, 64: 578–591.

BRUNSKILL, G.J., AND D.W. SCHINDLER. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139–155.

CARPENTER, S.R. 1989. Replication and treatment strength in whole-lake experiments. Ecology, 70: 453–463.

CARPENTER, S.R., AND A.M. BERGQUIST. 1985. Experimental tests of grazing indicators based on chlorophyll *a* degradation products. Arch. Hydrobiol. 102: 303–317.

CARPENTER, S.R., AND J.F. KITCHELL [ED.]. 1993. The trophic cascade in lake ecosystems. Cambridge University Press, Cambridge, Mass.

CARPENTER, S.R., J.F. KITCHELL, J.R. HODGSON, P.A. COCHRAN, J.J. ELSER, M.M. ELSER, D.M. LODGE, D. KRETCHMER, X. HE, AND C.N. VON ENDE. 1987. Regulation of lake primary productivity by food web structure. Ecology, 68: 1863–1876.

CARPENTER, S.R., P.R. LEAVITT, J.J. ELSER, AND M.M. ELSER. 1988. Chlorophyll budgets: response to food web manipulations. Biogeochemistry, 6: 79–90.

CHANG, P.S.S., D.F. MALLEY, W.J. FINDLAY, G. MUELLER, AND R.T. BARNES. 1980. Species composition and seasonal abundance of zooplankton in Lake 227, Experimental Lakes Area, northwestern Ontario, 1969–1978. Can. Data Rep. Fish. Aquat. Sci. 182.

CHANG, P.S.S., D.F. MALLEY, I.L DELBAERE, AND G. MUELLER. 1981. Species composition and seasonal abundance of zooplankton in Lake 223, Experimental Lakes Area, northwestern Ontario: before and during acidification, 1974–1979. Can. Data Rep. Fish. Aquat. Sci. 290.

CHENGALATH, R. 1982. A faunistic and ecological survey of the littoral Cladocera of Canada. Can. J. Zool. 60: 2668–2682.

CHENGALATH, R. 1987. The distribution of chydorid Cladocera in Canada. Hydrobiologia, 145: 151–157.

CLEUGH, T.R., AND B.W. HAUSER. 1971. Results of the initial survey of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 129–137.

DALEY, R.J. 1973. Experimental characterization of lacustrine chlorophyll diagenesis. II. Bacterial, viral and herbivore grazing effects. Arch. Hydrobiol. 72: 409–439.

DEAN, W.E. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. J. Sediment. Petrol. 44: 242–248.

DEEVEY, E.S. 1964. Preliminary account of fossilization of zooplankton in Rogers Lake. Int. Ver. Theor. Angew. Limnol. Verh. 15: 981–982.

DEEVEY, E.S., H. VAUGHAN, AND G.B. DEEVEY. 1977. Lakes Yaxha and Sacnab, Peten, Guatemala: planktonic fossils and sediment focusing, p. 189–196. *In* H.L. Golterman [ed.] Interactions between sediments and fresh water. Junk, The Hague.

ELSER, M.M., C.N. VON ENDE, P. SORANNO, AND S.R. CARPENTER. 1987. *Chaoborus* populations: response to food web manipulation and potential effects on zooplankton communities. Can. J. Zool. 65: 2846–2852.

FREY, D.G. 1988. Littoral and offshore communities of diatoms, cladocerans and dipterous larvae, and their interpretation in paleolimnology. J. Paleolimnol. 1: 179–192.

GOULDEN, C.E. 1971. Environmental control of the abundance and distribution of chydorid Cladocera. Limnol. Oceanogr. 16: 320–331.

GRIMM, E.C. 1987. CONISS: a Fortran 77 program for stratigraphically constrained cluster analysis by the method of incremental sum of squares. Comput. Geosci. 13: 13–35.

HANN, B.J. 1981. Occurrence and distribution of littoral Chydoridae (Crustacea, Cladocera) in Ontario, Canada and taxonomic notes on some species. Can. J. Zool. 59: 1465–1474.

HANN, B.J. 1989. Methods in Quaternary ecology. No. 6. Cladocera. Geosci. Can. 16: 17–26.

HANSEN, A.-M., J.V. CHRISTENSEN, AND O. SORTKJAER. 1991. Effect of high pH on zooplankton and nutrients in fish-free enclosures. Arch. Hydrobiol. 123: 143–164.

HOFMANN, W. 1986. Developmental history of the Grösser Plöner See and the Schöhsee (north Germany): cladoceran analysis, with special reference to eutrophication. Arch. Hydrobiol. Suppl. 74: 259–287.

HRBÁČEK, J. 1969. On the possibility of estimating predation pressure and nutrition level of populations of *Daphnia* from their remains in sediments. Mitt. Int. Ver. Theor. Angew. Limnol. 17: 262–274.

JOHNSON, M.G., J.R.M. KELSO, O.C. MCNEILL, AND W.B. MORTON. 1990. Fossil midge associations and historical status of fish in acidified lakes. J. Paleolimnol. 3: 113–127.

KERFOOT, W.C. 1974. Net accumulation rates and history of cladoceran communities. Ecology, 35: 384–398.

KERFOOT, W.C. 1975. Seasonal changes of *Bosmina* (Crustacea, Cladocera) in Frains Lake, Michigan: laboratory observations of phenotypic changes induced by inorganic factors. Freshwater Biol. 5: 227–243.

KERFOOT, W.C. 1981. Long-term replacement cycles in cladoceran communities: a history of predation. Ecology, 62: 216–233.

KERFOOT, W.C. 1987. Translocation experiments: *Bosmina* responses to copepod predation. Ecology, 68: 596–610.

KITCHELL, J.F. [ED.] 1992. Food web management: a case study of Lake Mendota. Springer-Verlag, New York.

KITCHELL, J.F., AND S.R. CARPENTER. 1987. Piscivores, planktivores, fossils and phorbins, p. 136–146. *In* W.C. Kerfoot and A. Sih [ed.] Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover, NH.

KITCHELL, J.F., AND P.R. SANFORD. 1992. Paleolimnological evidence of food web structure, p. 32–47. *In* J.F. Kitchell [ed.] Food web management: a case study of Lake Mendota. Springer-Verlag, New York.

LEAVITT, P.R., AND D.L. FINDLAY. 1994. Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci 51: 2286–2299.

LEAVITT, P.R., S.R. CARPENTER, AND J.F. KITCHELL. 1989. Whole-lake experiments: the annual record of fossil pigments and zooplankton. Limnol. Oceanogr. 34: 700–717.

LEAVITT, P.R., P.R. SANFORD, S.R. CARPENTER, J.F. KITCHELL, AND D. BENKOWSKI. 1993. Annual fossil records of food-web manipulation, p. 278–309. *In* S.R. Carpenter and J.F. Kitchell [ed.] The trophic cascade in lake ecosystems. Cambridge University Press, Cambridge, Mass.

LLOYD, M., AND R.J. GHELARDI. 1964. A table for calculating the 'equitability' component of species diversity. J. Anim. Ecol. 33: 217–225.

MAGURRAN, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J.

MALLEY, D.F., AND P.S.S. CHANG. 1980. Response of zooplankton in Precambrian Shield lakes to whole-lake chemical modifications causing pH change, p. 108–114. *In* U.S. Environmental Protection Agency [ed.] Restoration of lakes and inland waters. EPA 440/5-81-010.

MALLEY, D.F., P.S.S. CHANG, D.L. FINDLAY, AND G.A. LINSEY. 1988a. Extreme perturbation of the zooplankton community of a small Precambrian Shield lake by the addition of nutrients. Int. Ver. Theor. Angew. Limnol. Verh. 23: 2237–2247.

MALLEY, D.F., P.S.S. CHANG, AND D.W. SCHINDLER. 1988b. Decline of zooplankton populations following eutrophication of Lake 227, Experimental Lakes Area, Ontario: 1969–1974. Can. Tech. Rep. Fish. Aquat. Sci. 1619.

MCQUEEN, D.J., J.R. POST, AND E.L. MILLS. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. 43: 1571–1581.

NEILL, W.E., AND A. PEACOCK. 1980. Breaking the bottleneck: interactions of invertebrate predators and nutrients in oligotrophic lakes, p. 715–724.

In W.C. Kerfoot [ed.] Evolution and ecology of zooplankton communities. University Press of New England, Hanover, N.H.

O'Brien, W.J., and D. Schmidt. 1979. Arctic *Bosmina* morphology and copepod predation. Limnol. Oceanogr. 24: 564–568.

Patalas, K. 1971. Crustacean plankton communities in forty-five lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 231–244.

Pielou, E.C. 1977. Mathematical ecology. 2nd ed. John Wiley and Sons. New York.

Potts, W.T.W., and G. Fryer. 1979. The effects of pH and salt content on sodium balance in *Daphnia magna* and *Acantholeberis curvirostris* (Crustacea: Cladocera). J. Comp. Physiol. 129: 289–294.

Salo, J., M. Walls, M. Rajasilta, J. Sarvala, M. Rasanen, and V.P. Salonen. 1989. Fish predation and reduction in body size in a cladoceran population: palaeoecological evidence. Freshwater Biol. 21: 217–221.

Sanford, P.R. 1993. *Bosmina longirostris* antennule morphology as an indicator of intensity of planktivory by fishes. Bull. Mar. Sci. 53: 216–227.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes: natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. Science (Washington, D.C.), 195: 260–262.

Schindler, D.W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44 (Suppl 1): 6–25.

Schindler, D.W., F.A.J. Armstrong, S.K. Holmgren, and G.J. Brunskill. 1971. Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by the addition of phosphate and nitrate. J. Fish. Res. Board Can. 28: 1763–1782.

Schindler, D.W., H. Kling, R.V. Schmidt, J. Prokopowich, V.E. Frost, R.A. Reid, and M. Capel. 1973. Eutrophication of Lake 227 by addition of phosphorus and nitrate. Part 2. The second, third and fourth years of enrichment, 1970, 1971, 1972. J. Fish. Res. Board Can. 30: 1415–1440.

Schindler, D.W., K.G. Beaty, E.J. Fee, D.R. Cruikshank, E.R. DeBruyn, D.L. Findlay, G.A. Linsey, J.A. Shearer, M.P. Stainton, and M.A. Turner. 1990. Effects of climatic warming on lakes of the central boreal forest. Science (Washington, D.C.) 250: 967–970.

Sprules, W.G., J.C.H. Carter, and C.W. Ramcharan. 1984. Phenotypic associations in the Bosminidae (Cladocera): zoogeographic patterns. Limnol. Oceanogr. 29: 161–169.

Uutala, A.J. 1990. *Chaoborus* (Diptera: Chaoboridae) mandibles — paleolimnological indicators of the historical status of fish populations in acid sensitive lakes. J. Paleolimnol. 4: 139–152.

Whiteside, M.C. 1969. Chydorid (Cladocera) remains in surficial sediments of Danish lakes and their significance to paleolimnological interpretations. Mitt. Int. Ver. Theor. Angew. Limnol. 17: 193–201.

Whiteside, M.C. 1970. Danish chydorid Cladocera: modern ecology and core studies. Ecol. Monogr. 40: 79–118.

Whiteside, M.C. 1983. The mythical concept of eutrophication. Hydrobiologia, 103: 107–111.

Whiteside, M.C., and R.V. Harmsworth. 1967. Species diversity in chydorid (Cladocera) communities. Ecology, 48: 664–667.

Whiteside, M.C., and M.R. Swindoll. 1988. Guidelines and limitations to cladoceran paleoecological interpretations. Palaeogeogr. Palaeoclimatol. Palaeoecol. 62: 405–412.

Whiteside, M.C., J.B. Williams, and C.P. White. 1978. Seasonal abundance and pattern of chydorid Cladocera in mud and vegetative habitats. Ecology, 59: 1177–1188.

Wolfe, B., H.J. Kling, G.J. Brunskill, and P. Wilkinson. 1994. Multiple dating of a freeze core from Lake 227, an experimentally fertilized lake with varved sediments. Can. J. Fish. Aquat. Sci. 51: 2274–2285.

Yan, N.D., W. Keller, H.J. MacIsaac, and L.J. McEachern. 1991. Regulation of zooplankton community structure of an acidified lake by *Chaoborus*. Ecol. Appl. 1: 52–65.

Zeeb, B.A., C.E. Christie, J.P. Smol, D.L. Findlay, H.J. Kling, and H.J.B. Birks. 1994. Responses of diatom and chrysophyte assemblages in Lake 227 sediments to experimental eutrophication. Can. J. Fish. Aquat. Sci. 51: 2300–2311.

Paleolimnological analysis of whole-lake experiments: an overview of results from Experimental Lakes Area Lake 227[1]

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High resolution analysis of laminated sediments from experimentally fertilized Lake 227 was used to compare sediment geochemistry and fossil abundance (siliceous algae, pigments, cladoceran remains) with 20 yr (1969–1989) of continuous historical records. Members of all fossil groups were correlated to the biomass of their respective producer populations ($r = 0.52–0.66$, $P < 0.05$, $n = 20$). Correlations were greatest when fossil abundance was expressed per unit organic matter and least when calculated as the accumulation rate. Comparison of groups showed that fossils of soft-bodied zooplankton (copepods, rotifers) and pigments from dinoflagellates were completely unreliable. The most informative fossils were chitinous remains of zooplankton (e.g., *Bosmina*), remains of siliceous algae, and pigments from chlorophytes and cyanobacteria. Ecosystem-level paleolimnology showed that fertilization was the most significant eutrophication event in the last 400 yr and that it impacted both pelagic and littoral communities. However, fossil zooplankton and pigment analyses indicated that food-web interactions (zooplanktivory, herbivory) also regulated plankton abundance and composition. Additionally, fossil analyses showed that some natural eutrophication occurred prior to 1969 and that plankton communities have continued to vary since 1975, despite constant rates of fertilization with nitrogen and phosphorus.

On a eu recours à une analyse haute définition des sédiments laminés qui provenaient du lac 227, expérimentalement fertilisé, pour comparer la géochimie des sédiments et l'abondance des fossiles (algues siliceuses, pigments, restes de cladocères) avec des relevés historiques permanents qui couvrent 20 ans (1969–1989). On a établi une corrélation entre les membres de tous les groupes de fossiles et la biomasse des populations respectives qui les ont produits ($r = 0,52–0,66$, $P < 0,05$, $n = 20$). Les corrélations étaient les plus fidèles lorsque l'abondance des fossiles était exprimée en unités de matières organiques, et elles l'étaient le moins lorsqu'elles étaient calculées en terme de taux d'accumulation. La comparaison des groupes a montré que les fossiles d'espèces zooplanctoniques à corps mou (copépodes, rotifères) et que les pigments provenant des dinoflagellés ne sont pas fiables du tout. Ce sont les restes chitineux du zooplancton (p. ex. *Bosmina*), les restes d'algues siliceuses et les pigments de chlorophytes et de cyanobactéries qui se sont révélés être les fossiles les plus utiles. La paléolimnologie à l'échelle des écosystèmes a montré que la fertilisation a constitué l'événement le plus important, sur le plan de l'eutrophisation, au cours des 400 dernières années, et qu'elle a eu un effet sur les communautés pélagiques et littorales. Cependant, les analyses des pigments et du zooplancton fossile sont indiqué que les interactions trophiques (zooplanctophagie, phytophagie) ont aussi un effet de régulation sur l'abondance et la composition du plancton. De plus, les analyses des fossiles ont montré qu'il se produisait une certaine eutrophisation avant 1969 et que les communautés planctoniques n'ont pas cessé de varier depuis 1975 malgré l'application à taux constant du traitement de fertilisation à l'azote et au phosphore.

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Lake 227 has been experimentally fertilized since spring 1969. However, despite continuous chemical, biological, and meterological records during the ice-free seasons, little was known of the original aquatic food web. Consequently, extensive high-resolution paleolimnological investigations were initiated to study the effects of fertilization, reconstruct the premanipulation conditions, and compare fossil records against long-term, continuous data (Wolfe et al. 1994; Leavitt and Findlay 1994; Zeeb et al. 1994; Hann et al. 1994). This paper is a synthesis of the paleoecological studies.

Paleolimnology has the potential to provide historical information that is otherwise unavailable (Smol 1992). For example, premanipulation conditions in Lake 227 are known only from scattered surveys during 1967–1968, intense sampling early in 1969, and extrapolations from later regional surveys. Although the immediate effects of fertilization on plankton are well documented (Schindler et al. 1971, 1973; Schindler 1987), little is known of the long-term response of flora and fauna to eutrophication, particularly during the late 1970s and 1980s when sampling protocols changed and research effort was allocated to more urgent environmental issues such as acidification and toxic contamination. Similarly, benthic, littoral, and fish communities were rarely sampled and their responses to fertilization are unknown. The presence of undisturbed, continuously laminated sediments in Lake 227 made possible the use of high-resolution paleolimnology to extend the premanipulation data base, to investigate the importance of food-web interactions in controlling species replacements, and to examine the long-term effects of fertilization on the biota.

In this synthesis we have attempted to integrate the results from separate studies of sediment geochemistry (Wolfe et al. 1994), fossil pigments (Leavitt and Findlay 1994), algal microfossils (Zeeb et al. 1994), and cladoceran remains (Hann et al. 1994). In the first section, we compare different fossil groups and propose a hierarchy of reliability of fossils as paleolimnological indicators. In the second section, we focus on novel insights provided by ecosystem-level paleolimnology. In particular, we concentrate on events prior to fertilization and on the response of littoral communities and higher trophic levels to fertilization. This whole-lake approach is similar to those of other long-term studies (e.g., Schindler 1987; Kitchell 1992).

Methods

Site History

Lake 227, Experimental Lakes Area (ELA), Ontario, was first fertilized in June 1969, when phosphorus (P) inputs were increased 10-fold above natural levels to 0.56 g $P \cdot m^{-2} \cdot yr^{-1}$ (as sodium phosphate in 1969, and phosphoric acid thereafter). Nitrogen (N) was added in a 13:1 mass ratio with P (7.27 g $N \cdot m^{-2} \cdot yr^{-1}$ as sodium nitrate) between 1970 and 1974, but was reduced to a 5:1 ratio (3.3 g $N \cdot m^{-2} \cdot yr^{-1}$) between 1975 and 1989. N addition was discontinued in 1990. Lake and regional characteristics are detailed in Brunskill et al. (1971) and Schindler et al. (1973).

Plankton responses to fertilization were complex (Leavitt and Findlay 1994; Malley and Chang 1980; Malley et al. 1988). Original algal communities consisted of cryptophytes and chrysophytes in the pelagic zone (Schindler et al. 1973), and diatoms in the littoral zone (Stockner and Armstrong 1971). Zooplankton were mostly small-bodied crustaceans and included *Bosmina longirostris*, *Tropocyclops prasinus mexicanus*, and *Diaptomus minutus* (Patalas 1971; Malley et al. 1988). Fertilization increased the biomass of original algae, particularly chrysophytes, 10-fold by August, at which time green algae and cyanobacteria were most important. Chrysophytes benefitted from fertilization until 1972. In contrast, chlorophytes and filamentous cyanobacteria continued to increase until 1975 when N additions were reduced. N_2-fixing cyanobacteria became abundant after 1975, with *Aphanizomenon gracile* giving way to *Anabaena flos-aquae* (now *Aphanizomenon schindleri*; Kling et al. 1994) by 1982, the year of greatest phytoplankton biomass (Leavitt and Findlay 1994). Chlorophytes, filamentous cyanobacteria, and N_2-fixing cyanobacteria have remained common until the present time. Zooplankton communities initially benefitted from fertilization; however, epilimnetic pH reached a value of 10 during intense photosynthesis, and the abundance of Cladocera and copepods declined after 1973 (Malley and Chang 1980; Malley et al. 1988).

Sediment Analyses

Sediment cores were collected from deepwater locations in 1988 (Wolfe et al. 1994) and 1990 (Leavitt and Findlay 1994) using freeze-corers similar to those of Huttunen and Meriläinen (1978) and Swain (1973). Frozen sediments were partly freeze-dried and sectioned in approximately annual increments (1940 to present), using visible cycles of laminae as a guide to chronology (Leavitt and Findlay 1994; Wolfe et al. 1994). The presence of undulations in laminae, cyanobacterial mat intrusions, and subannual laminae (Wolfe et al. 1994) may have obscured annual cycles in the fertilization era, particularly from 1980–1990. However, patterns of fossil diatom abundance were compared between the 1988 and 1990 cores, and were used to establish a common sediment chronology.

Subsamples of approximately annual intervals were analyzed for sediment geochemistry and laminae fine structure (Wolfe et al. 1994), pigments (Leavitt and Findlay 1994), algal microfossils (Zeeb et al. 1994; Wolfe et al. 1994), and cladoceran remains (Hann et al. 1994). Details of procedures for fossil quantification are presented in the original papers. Instead, we summarize our techniques and present new data analyses.

Total P and N content was determined for annual increments in Wolfe's 1988 core between 1940 and 1977 following Stainton et al. (1977). Samples from 1979 ±1 yr and 1981 ±1 yr were also analyzed. For this paper, we also detrended P stratigraphies using first-difference procedures (Chatfield 1989). This technique removes long-term trends in time series and allows investigators to examine data for anomalously high or low values (Chatfield 1989).

All plankton fossils were quantified from the 1990 core (Leavitt and Findlay 1994). Algal pigments were extracted from whole sediments using organic solvents and were quantified by reversed-phase high performance liquid chromatography (Leavitt et al. 1989; Leavitt and Findlay 1994). Zooplankton were microscopically enumerated after dispersing fossils overnight in 10% KOH and concentrating cladoceran remains on a 53 μm mesh (Hann 1989; Hann et al. 1994). Sediments for recovery of siliceous algal microfossils were digested with a standard hot potassium dichromate and sulfuric acid mixture before enumeration for diatom

TABLE 1. Species richness in plankton and sediments for selected taxa in Lake 227, 1969–1989.

Group	Number of species		
	Plankton	Sediment	Plankton:sediment
Diatoms	17[a]	74	0.23
Chrysophytes	30[a]	45[b]	0.67
Scaled chrysophytes	8	16	0.50
Rotifers	11	0	—
Copepods	8[c]	0	—
Chaoborus	2	2[d]	1.0
Pelagic Cladocera	4	2[e]	2.0
Chydoridae	2[f]	22	0.09
Other zooplankton	1	7	0.14

[a]Two general groups (large, small) not included.
[b]Cyst morphotypes, 33 of which are known to species.
[c]Cyclopoids (5) and calanoids (3).
[d]Occasional mandibles only.
[e]Remains from two other species were not collected on the 53 μm screen.
[f]*Chydorus* and *Alona*, species unknown.

TABLE 2. Selected correlations (r) between volume-weighted annual plankton standing crop and fossil abundance. Plankton abundance measured as either $g \cdot m^{-2}$ (algae) or $individuals \cdot m^{-2}$ (*Bosmina*). Fossil β-carotene (as nmol pigment) and fossil *Bosmina* (as individuals) were expressed as $fossils \cdot (g\ organic\ matter)^{-1}$, $fossils \cdot (g\ dry\ weight)^{-1}$, and $fossils \cdot cm^{-2}$. Correlation significance: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Annual plankton standing crop	Fossil abundance		
	Per gram organic matter	Per gram dry weight	Per centimetre squared
Bosmina	0.66***	0.60**	0.21
β-carotene	0.65**	0.57**	0.29

and chrysophyte remains (Zeeb et al. 1994). Diatom and chrysophyte populations were estimated independently (1957–1989) as percent relative abundance (% fossil sum) because of the small aliquot size. All other estimates of annual fossil abundance were expressed three ways: (1) organic matter-specific concentration ($fossils \cdot [g\ organic\ matter]^{-1}$); (2) dry mass-specific concentration ($fossils \cdot [g\ dry\ weight]^{-1}$), and; (3) accumulation rate ($fossils \cdot cm^{-2}$). Organic content was estimated as weight loss on ignition (LOI; 500°C, 1 h; Dean 1974).

Correlations between plankton standing crop during the ice-free season ($individuals \cdot m^{-2}$ or $mass \cdot m^{-2}$) and fossil abundance (mass-specific concentration or accumulation rate) have been calculated for phytoplankton (Leavitt and Findlay 1994) and zooplankton (Hann et al. 1994). We calculated the relative abundance of the dominant postfertilization chrysophytes (*Mallomonas crassisquama*, *M. pseudocoronata*), and diatoms (Synedra PIRLA 2, *S. rumpens*, *S. rumpens* var. *familiaris*) independently for both fossil and plankton data sets. Pearson correlation coefficients (r) were compared among all fossil groups to evaluate the relative reliability of each group in recording planktonic events. Preliminary analysis showed that all fossil records, and most plankton records (except % *Mallomonas*, $P = 0.05$), were not significantly autocorrelated and that r values were reliable (Legendre 1993).

For this synthesis, plankton and fossil species richness was compared for the period 1969–1989. Nonsiliceous algae were not included because species composition of these phytoplankton communities cannot be estimated from fossil pigments and because algal resting stages are rare for most of these taxa. Ratios of plankton:fossil species richness were calculated to determine how well fossils represent water-column communities.

We also sought to reduce the complexity of the fossil data set by producing simple aggregations that were equivalent to functionally important ecological groups in Lake 227. For example, diatoms and chrysophytes were categorized as "acidophilic, unproductive, mesotrophic, benthic, and other" using published autecological literature (reviewed in Zeeb et al. 1994) and surface-sediment calibration data sets for the ELA (Findlay and Shearer 1992). The relative abundance of species within each ecological preference group was then summed for important taxa (i.e., those >10% of fossil sum) to estimate the relative abundance of the group. In addition, the size structure of pelagic zooplankton communities was estimated from fossil ratios of *Daphnia*:(*Daphnia* + *Bosmina*)(Hrbáček 1969; Leavitt et al. 1993). The ratio of chydorids:all Cladocera was used to approximate the relative importance of littoral taxa in Lake 227.

The magnitude of response to fertilization was quantified for all fossil groups by calculating a ratio of mean fossil abundance during 1970–1975 to that during 1950–1960. In addition, postfertilization variability was estimated for both fossils and plankton by calculating a coefficient of variation (CV = standard deviation/mean × 100) between 1970 and 1989.

Results

Plankton Preservation

Fossil groups differed in the degree to which they represented planktonic populations (Table 1). Soft-bodied copepods

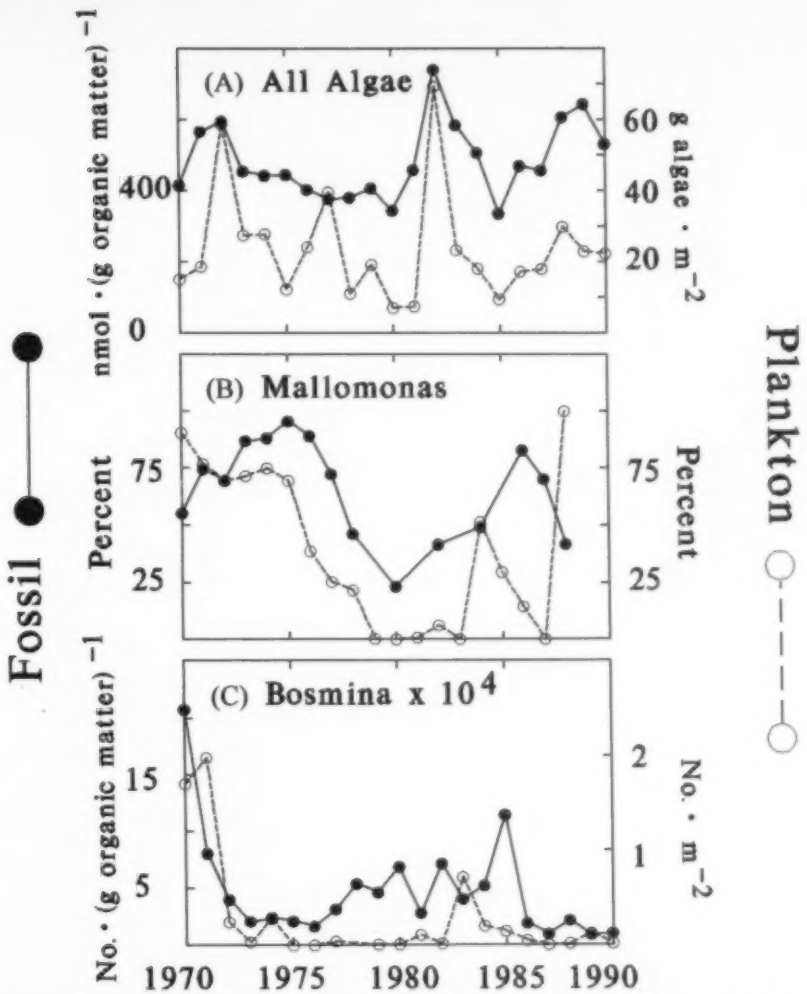

FIG. 1. Mean abundance of selected plankton and fossils in Lake 227, 1970–1989: (A) total algal biomass and fossil β-carotene concentration (from Leavitt and Findlay 1994); (B) relative abundance of *Mallomonas pseudocoronata* and *M. crassisquama* in planktonic and fossil chrysophyte assemblages (from Zeeb et al. 1994); and (C) *Bosmina longirostris* standing crop and fossil concentration (from Hann et al. 1994).

and rotifers were not preserved in sediments of Lake 227, and only two of four species of pelagic cladocerans were encountered in our samples (Hann et al. 1994). Although mandibles from both *Chaoborus punctipennis* and *C. flavicans* were collected, chaoborid remains were generally too rare to allow accurate estimation of annual population abundance. In contrast, many more taxa were recovered from sediments than were collected during field sampling for both diatoms and chydorids, two groups that include many species characteristic of the littoral zone. Also, sediments were better than plankton samples at representing scaled chrysophytes (*Mallomonas*) and rare Cladocera (e.g., *Polyphemus*; Table 1).

Relation between Fossils and Plankton

Members of all fossil groups (pigments, algae, zooplankton) were correlated to the biomass of respective producer populations between 1970 and 1989 (Fig. 1). Correlations were generally good for both pigments and animal microfossils (Table 2; Leavitt and Findlay 1994; Hann et al. 1994). In both cases, correlations were greatest when fossil abundance was expressed per unit organic matter and least when calculated as accumulation rate.

Some siliceous fossils were correlated with algal biomass, even though fossil and plankton abundance could only be calculated on a relative basis (Fig. 1; Zeeb et al. 1994). For example, plankton–fossil correlations were significant (r = 0.52; P = 0.02) for the sum of *Mallomonas crassisquama* + *M. pseudocoronata* between 1970 and 1988. In contrast, *Synedra* spp. were the dominant postfertilization diatoms in Lake 227, yet plankton and fossil abundances were uncorrelated. Unlike *Mallomonas*, *Synedra* are known to have significant benthic populations that are not sampled during plankton collections (Stockner 1971; Stockner and Armstrong 1971).

Estimates of plankton abundance were more variable than those based on fossil analyses (Table 3). Coefficients of

TABLE 3. Mean annual abundance and coefficient of variation (CV) for algal and zooplankton taxa measured from the plankton and fossil records of Lake 227, 1970–1989.

	Plankton[a]		Fossil[b]	
	Mean	CV	Mean	CV
Diatoms + chrysophytes	1 179	0.76	57	0.34
Cryptophytes	1 177	0.56	278	0.28
Chlorophytes	7 257	1.38	106	0.50
Chlorophytes + cyanobacteria	19 484	0.81	1 358	0.28
Filamentous cyanobacteria	11 967	1.16	254	0.33
N_2-fixing cyanobacteria	8 527	1.69	82	0.99
All cyanobacteria	12 227	1.13	193	0.27
Total algae	23 801	0.67	481	0.23
Bosmina	2 998	1.905	39 206	0.94
Daphnia[c]	—	—	9 184	0.73
Total chydorids[c]	—	—	28 484	0.48

[a]Algal biomass as $g \cdot m^{-2}$; zooplankton as individuals$\cdot m^{-2}$.
[b]Fossil pigments as nmol$\cdot$(g organic matter)$^{-1}$; fossil zooplankton as individuals$\cdot$(g organic matter)$^{-1}$.
[c]Complete field estimates not available.

variation for fossil pigments and fossil zooplankton were similar and were usually <50% of those estimated from plankton data. Amongst algae, the magnitude of the CV was inversely related to organism size in both fossil and plankton data sets: cryptophytes were the least variable phytoplankton, whereas large N_2-fixing cyanobacteria were the most variable. Variability also generally declined as taxa were aggregated into progressively larger groups (CVs: N_2-fixers > total cyanobacteria > cyanobacteria + green algae > total algae; Table 3). This pattern was particularly marked in the plankton data.

Fossil Record of Premanipulation Communities

Plankton communities showed subtle changes prior to nutrient addition. For example, concentrations of pigments from diatoms and chrysophytes (e.g. fucoxanthin) increased 25% after 1960 (Fig. 2A; Leavitt and Findlay 1994). Analysis of microfossils indicated that diatoms indicative of mesotrophic waters (e.g. *Synedra rumpens* and related taxa) were present in Lake 227 from 1960 onwards (Figs. 2B, 3A; Zeeb et al. 1994). Sedimentary P profiles from the 1988 core (Wolfe et al. 1994) generally paralleled the abundance of mesotrophic diatoms and exhibited both elevated P concentration during the 1960s and rapid increases after fertilization (Fig. 3B). However, detrended stratigraphies of sedimentary P suggested that elevated P concentrations during the 1960s were largely the result of a long-term trend towards increased P concentration in recent sediments, rather than short-term, abnormally high P content.

Animal communities also exhibited considerable variability before fertilization. *Bosmina* populations declined by 50% around 1960, leading to two-fold increases in the relative abundance of *Daphnia* (Fig. 2A). At the same time, total chydorid concentrations declined nearly 50% and acidophilic chydorids increased to their historical maximum abundance (Hann et al. 1994).

Fossil Record of Initial Fertilization (1969–1975)

Most paleoecological indicators showed marked changes between 1969 and 1972, coincident with the onset of experimental fertilization (Fig. 2). Concentrations of bulk sediment constituents (organic matter, N, P) increased 20–50% during 1970–1975 over mean fossil abundance during the 1950s (Table 4). In contrast, the concentrations of fossil pigments from green algae and cyanobacteria increased three- to five-fold when compared over the same two periods. Initial responses of the main zooplankton were more variable: *Bosmina* and chydorids declined, whereas *Daphnia* slightly increased (Hann et al. 1994). Zooplankton responses were similar in magnitude to those of bulk sediment characteristics and smaller than responses of primary producers (Table 4).

Plankton originally present in Lake 227 generally declined immediately after fertilization (Fig. 2A). Pigments derived from the original algal community of diatoms and chrysophytes (e.g., fucoxanthin) were reduced by 17%, and the main pelagic cladoceran *Bosmina longirostris* was reduced by >50% compared with abundances during the 1950s. In addition to reduced biomass, communities of siliceous algae also underwent striking species replacements (Fig. 2B). Diatoms and chrysophytes characteristic of unproductive, slightly acidic conditions (*Tabellaria flocculosa*, *Cyclotella stelligera*, *Aulacoseira distans* var. *tenella*, *Eunotia* PIRLA 1, *Mallomonas duerrschmidtiae*, *Synura sphagnicola*) dominated the algal microfossil assemblage from 1957–1969, but were replaced during 1970–1976 by diatoms typical of more productive waters (*Synedra* PIRLA 2, *S. rumpens* var. *familiaris*, *S. rumpens*) or generalist chrysophytes (*Mallomonas crassisquama*, *M. pseudocoronata*; Zeeb et al. 1994).

Littoral and benthic communities were also affected by the initial eutrophication of Lake 227. Relative abundances of both littoral chydorids and benthic diatoms increased until 1975, and then again after 1983 (Fig. 2). As occurred with diatoms (Zeeb et al. 1994), chydorids characteristic of more productive waters (e.g., *Chydorus brevilabris*) and sediment microhabitats (*Alona quadrangularis*, *A. circumfimbriata* Megard) replaced those typical of acidic waters (*A. intermedia*, *Disparalona acutirostris*, *Alonella excisa*) and phytophilous taxa (*C. faviformis*). Overall, the close correspondence of benthic diatoms and chydorids was maintained throughout the fertilization period.

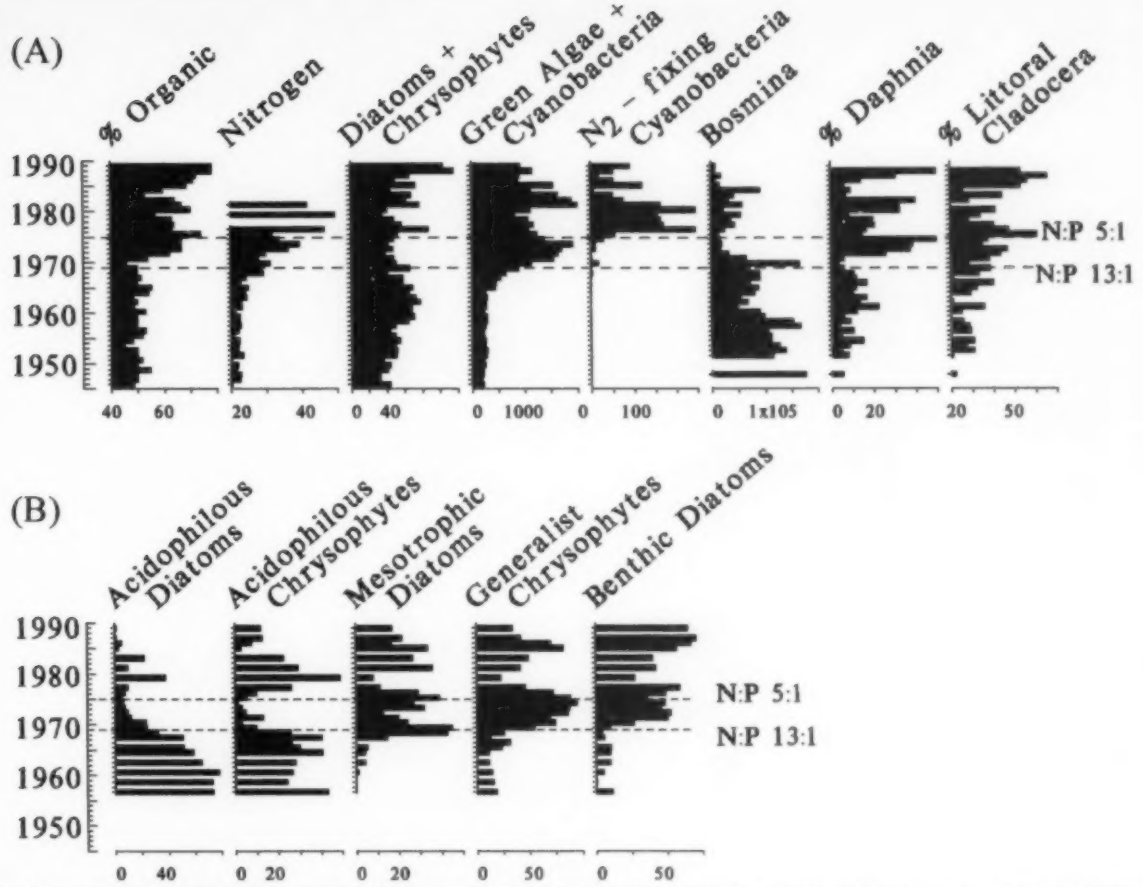

FIG. 2. Fossil stratigraphy from laminated sediments of Lake 227, 1945–1989. Lower dashed line in each panel = 1969, when nitrogen (N) and phosphorus (P) additions began at a 13:1 ratio (by mass); upper dashed line = 1975, when N and P were added at a 5:1 ratio. Panel A: % organic = % weight loss on ignition; nitrogen = mg N·(g dry weight)$^{-1}$ (1945–1983); diatoms + chrysophytes = fucoxanthin (nmol·[g organic matter]$^{-1}$); green algae (chlorophytes) + cyanobacteria = lutein–zeaxanthin (nmol·[g organic matter]$^{-1}$); N_2-fixing cyanobacteria = aphanizophyll (nmol·(g organic matter)$^{-1}$); *Bosmina* = individuals·(g organic matter)$^{-1}$; % *Daphnia* = *Daphnia*:(*Daphnia* + *Bosmina*); % littoral cladocera = chydorids:total Cladocera. Data from Leavitt and Findlay (1994; % organic, pigments), Wolfe et al. (1994; N), and Hann et al. (1994; invertebrates). Panel B: Relative abundance of siliceous phytoplankton (1957–1989), calculated independently for diatoms and chrysophytes (from Zeeb et al. 1994).

The size structure of the pelagic zooplankton community changed after the onset of experimental fertilization in 1969 (Fig. 2A; Hann et al. 1994). The abundance of large *Daphnia* relative to small *Bosmina* increased to a maximum of >50% in 1974. This peak resulted from both increased abundance of fossil *Daphnia* and declines in *Bosmina* (Hann et al. 1994). Large *Daphnia* spp. (carapace length >1 mm) were recorded in occasional hypolimnetic water samples during 1973–1974 (D. Malley and P. Chang, Dept. of Fisheries and Oceans, Winnipeg, Manitoba, unpublished data).

The length of time required to achieve new steady-state abundance after fertilization differed among fossil indicators. When compared with planktonic fossils, most bulk sediment indicators exhibited long lags between the onset of manipulations and final maximum concentration. Nitrogen and P concentrations (mg·[g dry wt]$^{-1}$) slowly increased to maxima in surface sediments, mirroring slow rises to steady state known from historical data (Schindler et al. 1987). Among bulk sediment indicators, only organic matter content (LOI) rapidly responded to known changes in lake conditions. In contrast, algal and animal responses were often complete in 2–3 yr, as measured either by fossil pigment concentration or by the abundance of microfossils (Fig. 2A).

Fossil Record of Reduced N Fertilization (1975–1989)

Reduced N input in 1975 was recorded by coincidental changes in most fossil markers (Fig. 2). Fossil pigments from N_2-fixing cyanobacteria replaced those from chlorophytes and other cyanobacteria as the main phytoplankton pigment during 1976–1982 (Leavitt and Findlay 1994). Overall, algal biomass inferred from fossil pigments and organic matter declined slowly after reduction in N inputs. Total algal biomass was low until 1979–1980 but increased rapidly to an historical maximum in 1982, when N_2-fixing cyanobacteria bloomed. Fossil pigments also indicated that chlorophytes and N_2-fixing cyanobacteria have continued to alternate as the dominant summer phytoplankton since 1983 (Fig. 2A).

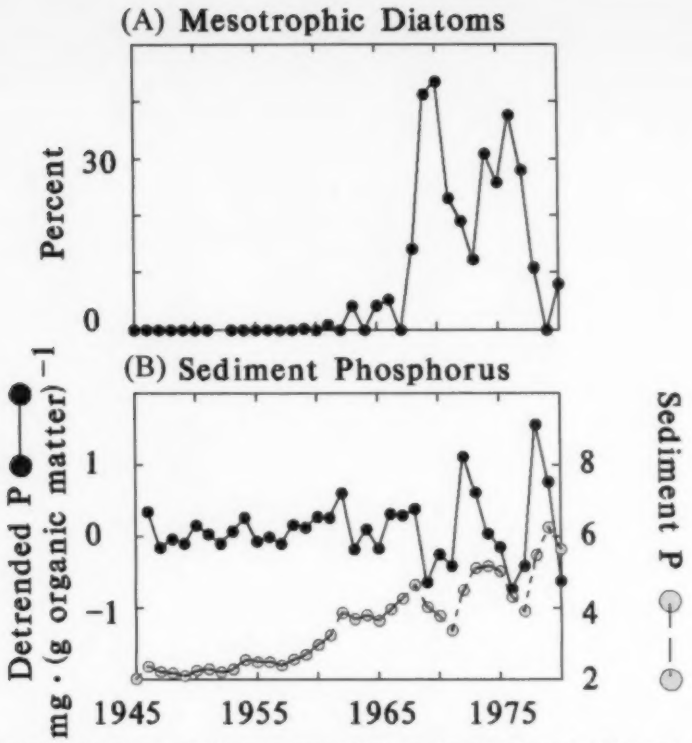

FIG. 3. Relative abundance of (A) mesotrophic diatoms (from Zeeb et al. 1994) and (B) sedimentary phosphorus (P) content (mg·[g dry weight]$^{-1}$; from Wolfe et al. 1994) in Lake 227, 1945–1980. Stippled dots = sediment P concentration, solid dots = detrended P profiles.

Several plankton groups originally present in the premanipulation assemblage recovered during the period when N_2-fixing cyanobacteria were common (Fig. 2). Both diatoms and chrysophytes characteristic of unproductive, acidic waters increased at the expense of mesotrophic indicator species and benthic diatoms. Similarly, the abundance of fossil *Bosmina* and *Daphnia* more than doubled during the late 1970s and early 1980s, whereas that of chydorids remained unchanged (Hann et al. 1994). However, these recoveries were not maintained, and native taxa once again declined during the late 1980s at the expense of mesotrophic, littoral, and benthic species (Fig. 2). According to fossil analysis, premanipulation communities of diatoms and *Bosmina* reached their historical minima during the most recent years.

Discussion

Accurate reconstruction of population change from sediment records requires reliable fossil estimates of plankton abundance. Our analyses showed that the sediments of Lake 227 only preserved a select subset of planktonic communities. However, if taxa were preserved, fossil abundance was significantly correlated with plankton standing stock, similar to results from other studies (e.g., Gorham et al. 1974; Haworth 1980; Guilizzoni et al. 1983). In fact, sediment estimates of algal and zooplankton abundance were less variable than those based on plankton samples (Table 3). Further, plankton–fossil correlations were equally strong among all fossil groups, thereby facilitating interpretation of population interactions. We feel our analyses are conservative because correlations were calculated only during the fertilization era, and missed most of the initial large response to eutrophication (Fig. 2). In addition, plankton–fossil correlations may have been much stronger had plankton been collected year-round and with uniform techniques (Leavitt and Findlay 1994; Hann et al. 1994).

Applicability to Other Lakes

In many respects, Lake 227 is an ideal system for calibrating fossils both because of 20 yr of historical data, and also because of laminated sediments that facilitate estimation of sediment age. Unfortunately, most other lakes lack one or both of these factors. Consequently, it is useful to identify general patterns in our analyses that should be applicable to other lakes.

Choice of Fossil Group

Not all plants and animals leave reliable fossil records (Table 1), so we have attempted to rank fossil groups according to their ability to record historical changes in abundance of chemicals or plankton in the water column.

Unreliable
Dinoflagellate carotenoids
Nonchitinous zooplankton (copepods, rotifers)
Sediment accumulation rate

Unknown reliability
Carotenoids from cryptophytes, siliceous algae

TABLE 4. Mean abundance of bulk sediment characteristics, fossil algal pigments, and fossil zooplankton prior to manipulation (= Pre: 1950–1960), and during years of high nitrogen (N) and phosphorus (P) addition (= Post: 1970–1975).

	Mean abundance		
	1950–1960	1970–1975	Post:pre
Bulk sediments			
Organic[a]	50.24	60.31	1.20
Carbon[b]	240.46	296.83	1.23
Mass accumulation[c]	5.78	8.02	1.39
Organic accumulation[c]	3.19	4.89	1.53
N[b]	21.07	32.47	1.54
P[b]	3.10	5.12	1.65
Algal pigments			
Siliceous algae (fucoxanthin)[d]	53.38	45.59	0.85
Cryptophytes (alloxanthin)[d]	160.41	285.12	1.78
All cyanobacteria (echinenone)[d]	44.40	150.64	3.39
All algae (β-carotene)[d]	137.91	486.10	3.52
Colonial cyanobacteria (myxoxanthophyll)[d]	78.83	280.67	3.56
Chlorophytes + cyanobacteria (lutein-zeaxanthin)[d]	260.14	1553.88	5.97
Zooplankton Fossils			
Daphnia[e]	9.604×10^3	1.001×10^4	1.05
Chydoridae[e]	4.203×10^4	3.414×10^4	0.81
Bosmina[e]	1.109×10^5	5.265×10^4	0.47

[a]Relative %.
[b]mg·(g dry weight)$^{-1}$.
[c]mg·(cm^2·yr^1)$^{-1}$.
[d]nmol pigment·(g organic matter)$^{-1}$.
[e]Individuals·(g organic matter)$^{-1}$.

Reliable
Phosphorus and nitrogen content
Organic content (loss on ignition)

Most reliable
Algal microfossils
Chitinous Cladocera (e.g., *Bosmina*)
Carotenoids from chlorophytes and cyanobacteria
Ubiquitous pigments (e.g., β-carotene, pheophytin *a*)

Soft-bodied zooplankton (copepods, rotifers, etc.) were absent from the fossil record (Hann et al. 1994), as were pigments from dinoflagellate algae (Leavitt and Findlay 1994). Typically, both groups are represented in sediments by resting cysts or reproductive structures (Frey 1988), but the relation between population size and fossil cyst abundance is unknown. Similarly, carotenoids from cryptophytes and siliceous algae were only weakly correlated to algal standing crops, either because of selective preservation or because of inadequate plankton sampling during the winter (Leavitt and Findlay 1994). Fossil pigments from diatoms and chrysophytes are greatly influenced by conditions during preservation (e.g., Leavitt and Carpenter 1990a, 1990b; Hurley and Armstrong 1990, 1991) and should be interpreted cautiously.

Bulk-sediment indicators were relatively insensitive to ecosystem change. Total sediment and organic matter accumulation rates, and percent organic matter (as LOI) were uncorrelated to changes in algal biomass (Leavitt and Findlay 1994; P.R. Leavitt and D.L. Findlay, unpublished data), despite the observation that organic content paralleled known changes in algal biomass (Fig. 2A). Also, bulk-sediment indicators increased relatively little (<60%; Table 4) in comparison to the four- to 10-fold increases in algal biomass known from fossil records (Fig. 2A) and scattered historical data (Schindler et al. 1973). Taken together, poor plankton–fossil correlations and low sensitivity to eutrophication suggest that much of the sediment matrix may be derived from nonplanktonic sources. We are currently investigating the use of stable isotopes in Lake 227 sediments to determine the relative contribution of autochthonous production to total sediment deposition.

Biogeochemical markers were more difficult to evaluate because of incomplete fossil and water-column records. Sedimentary N and P concentrations increased until 1974 and again after 1977 (Fig. 2A; N only), and generally paralleled whole-lake N and P content until 1982 (Schindler et al. 1987). As occurred with other bulk-sediment indicators, concentrations of sediment N and P increased less than two-fold (Table 4). Although mass budgets were not available prior to 1969, estimates based on regional lake surveys suggest that whole-lake P input increased >10-fold with fertilization, whereas N content increased more than five-fold

(Schindler et al. 1987). Again, these data are consistent with a high flux of nonplanktonic matter to the sediments.

The most reliable fossil indicators were hard fossils from plankton, ubiquitous pigments (β-carotene and pheophytin *a*), and carotenoids from green algae and cyanobacteria (Hann et al. 1994; Leavitt and Findlay 1994; Zeeb et al. 1994). All groups showed moderately strong fossil–plankton correlations ($r > 0.5$, $P < 0.05$), despite changes in historical sampling protocols, incomplete plankton sampling (no winter or littoral samples), incomplete isolation of adjacent sediment samples, and potential difficulties identifying annual laminae (Wolfe et al. 1994). Pigment correlations were probably good because chlorophytes and cyanobacteria exhibited a wide range of standing stocks and dominated biomass during the ice-free season, when plankton sampling was most intense (Leavitt and Findlay 1994). Biomass–fossil correlations with the dominant diatoms were generally not significant (e.g., *Synedra*), probably because most diatoms in Lake 227 have benthic populations (Stockner 1971; Stockner and Armstrong 1971) that are not sampled by normal plankton methods. The importance of incomplete sampling was shown by analyses of *Mallomonas* spp., chrysophytes that do not have benthic phases. Correlations between planktonic *Mallomonas pseudocoronata* + *M. crassisquama* and their fossil scales were significant ($r = 0.52$, $P < 0.05$), despite being based on relative abundance calculations. Although the small size of sediment aliquots prevented accurate estimation of algal microfossil concentrations (Zeeb et al. 1994), the significant correlation between planktonic and fossil *Mallomonas* confirmed that standard relative abundance diagrams accurately record complex community change resulting from eutrophication.

Choice of Sampling Interval

Our analyses suggest that choice of sediment sampling interval depends on the process being measured. Slow processes, such as the increase in N or P content of Lake 227 (Figs. 2A, 3B), did not require annual resolution because the actual approach to a steady state required years (Schindler et al. 1987). In contrast, quantification of rapid plankton community change benefitted from high-resolution analyses.

Annual resolution was difficult to achieve despite the presence of distinct, and often annual, laminae in the sediments. Several years during the fertilization era had subannual laminae, algal-mat intrusions, and indistinct layers that obscured annual cycles and made it difficult to isolate annual increments of sediment. In addition, we found that investigators differed in their identification of annual cycles. Furthermore, manual isolation of annual increments was often incomplete and invariably led to some mixing between adjacent samples. Despite these difficulties, good correlations between field and fossil data suggest that estimates of sediment age were rarely in error.

Accurate reconstruction of plankton abundance in individual years was difficult because of high variability in both fossil and plankton records (Table 3). Zooplankton and highly specific algal groups (e.g., N_2-fixing cyanobacteria) were particularly variable. Some plankton variability may be caused by changes in sampling techniques. Phytoplankton have been collected using at least four distinctive sets of sampling gear and sampling strategies in the last 20 yr (Findlay and Kasian 1987), and rarely included samples from beneath permanent ice cover or from littoral communities. Similarly, zooplankton populations have been analyzed at several intervals (weeks to months) and are known to be variable (Malley and Chang 1980; Malley et al. 1988). Consequently, because of both high plankton variability and the difficulty in completely isolating annual laminae, we feel that accurate estimation of a single year's plankton abundance from the fossil record is extremely difficult. Instead, when high-resolution studies are required, investigators should concentrate on fossil signals that last several years and that are easily distinguished from background noise.

Choice of Units

Only mass-specific fossil concentration was correlated with plankton biomass. As pointed out by Leavitt and Findlay (1994), a lack of correlation between fossil accumulation rates and plankton abundance may arise because of difficulties in accurately estimating annual bulk sediment accumulation. However, accumulation rates are also difficult and expensive to quantify in postglacial analyses and are often interpolated between samples separated by $10^2 - 10^3$ yr. Because organic content (LOI) and sediment weight are simple, inexpensive, and accurate to determine, we feel that investigators should interpret fossil stratigraphies using both fossil concentration and accumulation rate.

Correlations between plankton and fossils were best when fossil abundance was expressed per unit organic matter (Table 2; Leavitt and Findlay 1994; Hann et al. 1994). We suggest that organic-specific concentrations are superior to dry mass-specific concentrations as fossil measures because they are less sensitive to artifacts arising from fossil degradation and nonplanktonic influx of inorganic matter. For example, algal pigments (Leavitt and Carpenter 1990a, 1990b; Hurley and Armstrong 1990, 1991), *Bosmina* exuviae (Kerfoot 1981; Leavitt et al. 1993), and total organic matter (Depinto 1977; Fallon and Brock 1980; Parkin and Brock 1981) are all substantially degraded before deposition. Compensatory losses of bulk organic matter may partly balance losses of fossils during deposition (e.g., Hurley et al. 1992), and organic matter-specific concentrations may remain constant. In contrast, concentrations expressed in terms of dry weight are less affected by organic matter loss and remain more biased. Similarly, deposition of inorganic material can be sensitive to changes in chemical equilibria (e.g., calcium carbonate precipitation, salt crystallization, etc.) that may alter fossil concentration per unit dry weight independent of plankton production.

Novel Insights from Ecosystem-Level Paleolimnology

Historical Significance of Fertilization

Our analysis showed that experimental fertilization was the largest eutrophication event in Lake 227 in the last 400 yr, dwarfing even forest fires (Leavitt and Findlay 1994; Wolfe et al. 1994). Paleolimnological records documented that postfertilization communities were unlike any known from regional surveys or the premanipulation period (Schindler and Holmgren 1971). Fossil pigments showed that algal biomass increased approximately four-fold (Fig. 2A), somewhat lower than the 10-fold estimates based on sporadic field samples during 1968–1969 (Leavitt and Findlay 1994). Analysis of algal microfossils also detailed massive species replacements in diatom and chrysophyte communities (Fig. 2B), events that were unknown from historical records (Zeeb et al. 1994).

Fossil analyses showed that Lake 227 also underwent significant change prior to the onset of experimental fertilization (Figs. 2, 3). Pigments and microfossils of mesotrophic diatoms increased around 1960, coincident with increased sedimentary P content and reduced abundance of *Bosmina* and chydorids. All events are consistent with patterns observed during experimental fertilization, and suggest that some natural eutrophication occurred during the 1960s. Hann et al. (1994) have suggested that Lake 227 is subject to periodic winterkills of fish and, perhaps, invertebrates. Prolonged anaerobic conditions in spring may have increased internal nutrient flux to the epilimnion and, in turn, algal production. Lake 227 is naturally colored, strongly stratified, and dominated by small cyprinids, a combination of traits that is characteristic of winterkill lakes.

Postfertilization Events

Fossil analyses accurately recorded many postfertilization species replacements known from water sample analyses, including loss of *Bosmina*, increases in chlorophytes and cyanobacteria, and establishment of N_2-fixing cyanobacteria after 1975 (Fig. 2A). Fossil analyses also detailed changes in diatom, chrysophyte, and chydorid populations that were inadequately sampled by conventional methods. In addition, ecosystem-level analyses revealed several hitherto unknown responses to eutrophication.

Paleolimnological analyses suggested that food-web interactions helped regulate plankton abundance in Lake 227, even after 10-fold increases in P inputs. Hann et al. (1994) showed that fossil *Bosmina* were small-bodied and that *Daphnia* were rare between 1969 and 1973 (Fig. 2A). However, both the relative abundance of *Daphnia* and *Bosmina* body size increased to near historical maxima in 1974, coincident with spring winterkill and the appearance of large *Daphnia* (>1 mm) and *Chaoborus* in deepwater plankton samples (Malley et al. 1988). Although historical records of fish populations are not available, these changes in the size structure of zooplankton are consistent with reduced predation by fish after winterkill. Size-selective vertebrate zooplanktivory usually both eliminates larger invertebrates and reduces the mean size of those adult prey that remain (e.g., Hrbáček 1969; Kerfoot 1974; Leavitt et al. 1993).

Food-web interactions may also have affected the formation of the fossil record in Lake 227. Pigments in premanipulation sediments were biased towards alloxanthin, a carotenoid whose deposition is closely controlled by herbivory (Leavitt et al. 1989, 1993). Furthermore, alloxanthin concentrations were strongly correlated to those of pheophorbide *a* ($r = 0.76$, $P < 0.001$, $n = 39$; Leavitt and Findlay 1994), a grazing indicator that was itself correlated ($r = 0.64$, $P < 0.001$) to the abundance of large *Daphnia* in the plankton (Hann et al. 1994). The abundance of large *Daphnia* relative to small *Bosmina* is an index of total herbivory by all large invertebrates (Salo et al. 1989; Leavitt et al. 1989, 1993). Large herbivores harvest a wider size range of algae (Burns 1968) and are more effective at depositing algal pigments than are smaller Cladocera (Carpenter et al. 1986, 1988; Leavitt et al. 1989).

Analysis of both algal and zooplankton fossils demonstrated that littoral and benthic communities were significantly impacted by fertilization (Fig. 2). Chydorids associated with vegetation declined in favor of species that prefer sediments or cyanobacterial-mat habitats (Hann et al. 1994). Similarly, benthic diatoms benefitted from eutrophication relative to planktonic species (Zeeb et al. 1994), although it is unclear whether there was a net increase in onshore diatom production. We speculate that sediments may have buffered the most extreme changes in water chemistry (e.g., pH; Malley et al. 1988) and promoted populations of littoral taxa. Unfortunately, historical data for the littoral zone are rare.

Finally, high-resolution paleolimnology demonstrated that plankton communities have continued to vary, despite constant nutrient inputs from 1975–1990. Acidophilic diatoms, chrysophytes, and *Bosmina* populations that were present originally all partly recovered coincident with the high abundance of N_2-fixing cyanobacteria between 1975 and 1983 (Fig. 2). However, this recovery was short-lived and, after 1984, cyanobacteria gave way to chlorophytes, *Bosmina* declined, and productive diatoms and chrysophytes again replaced acidophilous taxa. The changes in N_2-fixing cyanobacteria closely parallelled observed alterations in the whole-lake N:P ratio (Schindler et al. 1987; B. Parker, Dept. of Zoology, University of Alberta, Edmonton, AB, unpublished data). Naturally high N levels during 1982–1987 may have reduced the relative success of N_2-fixing cyanobacteria. However, it is unclear how these chemical changes would influence cladoceran or diatom communities.

In summary, comparison of 20 yr of historical plankton records with annual fossil profiles showed that sediments selectively recorded planktonic communities. Soft-bodied zooplankton and pigments from dinoflagellates were not preserved. In contrast, sediment samples were better than plankton samples at representing diatom and chydorid communities, two groups with significant littoral or benthic populations. In cases where fossils could be accurately quantified, we found that mass-specific fossil concentration was significantly correlated to plankton biomass. Correlations were equally good for algal and invertebrate taxa. Surprisingly, fossil accumulation rates were uncorrelated with plankton standing crop. Historical reconstructions using all fossil groups provided information that was otherwise unavailable. For example, fossils showed that, although experimental fertilization was the most significant eutrophication in the last 400 yr, zooplankton communities exhibited natural variability of a magnitude similar to that resulting from eutrophication. Similarly, algal microfossils and pigments showed that phytoplankton communities remained variable, even under constant nutrient input regimes. Comparison of fossil zooplankton and pigments indicated that food-web interactions (predation, herbivory) may also regulate planktonic populations and can contribute to long-term variability.

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References

Brunskill, G.J., D. Povoledo, B.W. Graham, and M.P. Stainton. 1971. Chemistry of surface sediments of sixteen lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 277–294.

BURNS, C.W. 1968. The relationship between body size of filter-feeding Cladocera and the maximum particle size ingested. Limnol. Oceanogr. 13: 675–678.

CARPENTER, S.R., M.M. ELSER, AND J.J. ELSER. 1986. Chlorophyll production, sedimentation and degradation: implications for paleolimnology. Limnol. Oceanogr: 31: 112–124.

CARPENTER, S.R., P.R. LEAVITT, J.J. ELSER, AND M.M. ELSER. 1988. Chlorophyll budgets: response to food web manipulations. Biogeochemistry 6: 79–90.

CHATFIELD, C. 1989. The analysis of time series. 4th ed. Chapman and Hall, New York.

DEAN, W.E. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. J. Sediment. Petrol. 44: 242–248.

DEPINTO, J.V. 1977. Water column death and decomposition of phytoplankton: an experimental and modelling review, pp. 25–52. *In* D. Scavia and A. Robertson [eds.] Lake ecosystem modelling. Ann Arbor Science, Ann Arbor, MI.

FALLON, R.D., AND T.D. BROCK. 1980. Planktonic blue-green algae: production, sedimentation and decomposition in Lake Mendota, WI. Limnol. Oceanogr. 25: 72–88.

FINDLAY, D.L., AND S.E.M. KASIAN. 1987. Phytoplankton community responses to nutrient addition in Lake 226, Experimental Lakes Area, northwestern Ontario. Water Air Soil Pollut. 30: 719–726.

FINDLAY, D.L., AND J.A. SHEARER. 1992. Relationships between sedimentary diatom assemblages and lakewater pH values in the Experimental Lakes Area. J. Paleolimnol. 7: 157–162.

FREY, D.G. 1988. Littoral and offshore communities of diatoms, cladocerans and dipterous larvae, and their interpretation in paleolimnology. J. Paleolimnol. 1: 179–192.

GORHAM, E., J.W.G. LUND, J.E. SANGER, AND W.E. DEAN, JR. 1974. Some relationships between algal standing crop, water chemistry, and sediment chemistry in the English Lakes. Limnol. Oceanogr. 19: 601–617.

GUILIZZONI, P., G. BONOMI, G. GALANTI, AND D. RUGGIU. 1983. Relationship between sedimentary pigments and primary production: evidence from core analyses of twelve Italian lakes. Hydrobiologia, 103: 103–106.

HANN, B.J. 1989. Methods in Quaternary ecology. No. 6. Cladocera. Geosci. Can. 16: 17–26

HANN, B.J., P.R. LEAVITT, AND P.S.S. CHANG. 1994. Cladocera community response to experimental eutrophication in Lake 227 as recorded in laminated sediments. Can. J. Fish. Aquat. Sci. 51: 2312–2321.

HAWORTH, E. Y. 1980. Comparison of continuous phytoplankton records with the diatom stratigraphy in the recent sediments of Blelham Tarn. Limnol. Oceanogr. 25: 1093–1103.

HRBÁČEK, J. 1969. On the possibility of estimating predation pressure and nutrition level of populations of *Daphnia* from their remains in sediments. Mitt. Int. Ver. Theor. Angew. Limnol. 17: 262–274.

HURLEY, J.P., AND D.E. ARMSTRONG. 1990. Fluxes and transformations of aquatic pigments in Lake Mendota, Wisconsin. Limnol. Oceanogr. 35: 384–398.

HURLEY, J.P., AND D.E. ARMSTRONG. 1991. Pigment preservation in lake sediments: a comparison of sedimentary environments in Trout Lake, Wisconsin. Can. J. Fish. Aquat. Sci. 48: 472–486.

HURLEY, J.P., D.E. ARMSTRONG, AND A. L. DUVALL. 1992. Historical interpretation of pigment stratigraphy in Lake Mendota sediments. pp. 49–68. *In* J.F. Kitchell [ed.] Food web management: a case study of Lake Mendota. Springer–Verlag, New York.

HUTTUNEN, P., AND J. MERILÄINEN. 1978. New freezing device providing large unmixed sediment samples from lakes. Ann. Bot. Fenn. 15: 128–130.

KERFOOT, W.C. 1974. Net accumulation rates and history of cladoceran communities. Ecology, 35: 384–398.

KERFOOT, W.C. 1981. Long-term replacement cycles in cladoceran communities: a history of predation. Ecology, 62: 216–233.

KITCHELL, J.F. [ed]. 1992. Food web management: a case study of Lake Mendota. Springer–Verlag, New York.

KLING, H.J., D.L. FINDLAY, AND J. KOMAREK. 1994. *Aphanizomenon schindleri* sp.nov.: a new nostocacean cyanoprokaryote from the Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 51: 2267–2273.

LEAVITT, P.R., AND S.R. CARPENTER. 1990a. Aphotic pigment degradation in the hypolimnion: implications for sedimentation studies and paleolimnology. Limnol. Oceanogr. 35: 520–535.

LEAVITT, P.R., AND S.R. CARPENTER. 1990b. Regulation of pigment sedimentation by photo-oxidation and herbivore grazing. Can. J. Fish. Aquat. Sci. 47: 1166–1176.

LEAVITT, P.R., AND D.L. FINDLAY. 1994. Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci. 51: 2286–2299.

LEAVITT, P.R., S.R. CARPENTER, AND J.F. KITCHELL. 1989. Whole-lake experiments: the annual record of fossil pigments and zooplankton. Limnol. Oceanogr. 34: 700–717.

LEAVITT, P.R., P.R. SANFORD, S.R. CARPENTER, J.F. KITCHELL, AND D. BENKOWSKI. 1993. Annual fossil records of food-web manipulation, pp. 278–309. *In* S.R. Carpenter and J.F. Kitchell [ed.] The trophic cascade in lakes. Cambridge University Press, Cambridge, Mass.

LEGENDRE, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology, 74: 1659–1673.

MALLEY, D.F., AND P.S.S. CHANG. 1980. Response of zooplankton in Precambrian Shield lakes to whole-lake chemical modifications causing pH change, pp. 108–114. *In* U.S. Environmental Protection Agency [ed.] Restoration of lakes and inland waters. EPA 440/5-81-010.

MALLEY, D.F., P.S.S. CHANG, D.L. FINDLAY, AND G.A. LINSEY. 1988. Extreme perturbation of the zooplankton community of a small Precambrian Shield lake by the addition of nutrients. Int. Ver. Theor. Angew. Limnol. Verh. 23: 2237–2247.

PARKIN, T.B., AND T.D. BROCK. 1981. Photosynthetic production and carbon mineralization in a meromictic lake. Arch. Hydrobiol. 91: 366–382.

PATALAS, K. 1971. Crustacean plankton communities in forty-five lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 231–244.

SALO, J., M. WALLS, M. RAJASILTA, J. SARVALA, M. RASANEN, AND V.P. SALONEN. 1989. Fish predation and reduction in body size in a cladoceran population: palaeoecological evidence. Freshwater Biol. 21: 217–221.

SCHINDLER, D.W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 6–25.

SCHINDLER, D.W., AND S.K. HOLMGREN. 1971. Primary production and phytoplankton in the Experimental Lakes Area, northwestern Ontario, and other low-carbonate waters, and a liquid scintillation method, for determining ^{14}C activity in photosynthesis. J. Fish. Res. Board Can. 28: 189–201.

SCHINDLER, D.W., F.A.J. ARMSTRONG, S.K. HOLMGREN, AND G.J. BRUNSKILL. 1971. Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by the addition of phosphate and nitrate. J. Fish. Res. Boârd Can. 28: 1763–1782.

SCHINDLER, D.W., H. KLING, R.V. SCHMIDT, J. PROKOPOWICH, V.E. FROST, R.A. REID, AND M. CAPEL. 1973. Eutrophication of Lake 227 by addition of phosphorus and nitrate. Part 2. The second, third and fourth years of enrichment, 1970, 1971, 1972. J. Fish. Res. Board Can. 30: 1415–1440.

SCHINDLER, D.W., R.H. HESSLEIN, AND M.A. TURNER. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 26–33.

SMOL, J.P. 1992. Paleolimnology: an important tool for effective ecosystem management. J. Aquat. Ecosyst. Health, 1: 49–58.

STAINTON, M.P., M.L. CAPEL, AND F.A.J. ARMSTRONG. 1977. The chemical analysis of fresh water. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25.

STOCKNER, J.G. 1971. Preliminary characterization of lakes in the Experimental Lakes Area, northwestern Ontario, using diatom occurrences in sediments. J. Fish. Res. Board Can. 28: 265–275.

STOCKNER, J.G., AND F.A.J. ARMSTRONG. 1971. Periphyton of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 215–226.

SWAIN, A.M. 1973. A history of fire and vegetation in northeastern Minnesota as recorded in lake sediments. Quat. Res. 3: 383–396.

WOLFE, B., H.J. KLING, G.J. BRUNSKILL, AND P. WILKINSON. 1994. Multiple dating of a freeze core from Lake 227, an experimentally fertilized lake with varved sediments. Can. J. Fish. Aquat. Sci. 51: 2274–2285.

ZEEB, B.A., C.E. CHRISTIE, J.P. SMOL, D.L. FINDLAY, H.J. KLING, AND H.J.B. BIRKS. 1994. Responses of diatom and chrysophyte assemblages in Lake 227 sediments to experimental eutrophication. Can. J. Fish. Aquat. Sci. 51: 2300–2311.

PERSPECTIVES

Determination of Fish Age Using ^{210}Pb : ^{226}Ra Disequilibrium Methods

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West, I.F., and R.W. Gauldie. 1994. Determination of fish age using ^{210}Pb : ^{226}Ra disequilibrium methods. Can J. Fish Aquat. Sci. 51: 2333–2340.

Previous workers have used disequilibrium in the ^{210}Pb : ^{226}Ra ratio in fish otoliths to estimate the age of four teleost species: *Hoplostethus atlanticus, Macruronus novaezelandiae, Sebastes diploproa,* and *Sebastes mentella.* We argue that uncontrollable errors due to degassing of intermediate ^{222}Rn from otoliths, unknown sources and sinks of ^{210}Pb and ^{226}Ra, possible changes in the ratio with which these isotopes are accreted to the otolith over the life of the fish, contamination by ^{210}Po, difficulties in obtaining otolith cores, and the dependence of the estimated radiometric ages on the mass growth-in-time model usually adopted for otoliths can invalidate the application of disequilibrium techniques to fish age estimation.

Des chercheurs ont appliqué les déséquilibres observés dans le rapport entre le ^{210}Pb et le ^{226}Ra contenus dans les otolithes de poisson afin d'estimer l'âge chez quatre espèces de téléostéens : *Hoplostethus atlanticus, Macruronus novaezelandiae, Sebastes diploproa* et *Sebastes mentella.* Nous faisons valoir que des erreurs incontrôlables et qui sont attribuables à la libération du ^{222}Rn (un composé intermédiaire) des otolithes, des sources et des puits inconnus de ^{210}Pb et de ^{226}Ra, de possibles variations de la vitesse à laquelle ces isotopes s'accumulent dans les otolithes au cours de la vie du poisson, la contamination par le ^{210}Po, la difficulté d'obtenir des microcarottes prélevées dans des otolithes, ainsi que le lien de dépendance des âges radioisotopiques vis-à-vis le modèle de croissance en masse dans le temps auquel on a ordinairement recours avec les otolithes, peuvent rendre invalide l'application des techniques fondées sur les déséquilibres des rapports de concentration pour estimer l'âge de poissons.

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One of the fundamental problems in using otoliths to age fishes is that the various patterns by which age is assigned are themselves products of the growth pattern of the otolith, and hence of the fish (Gauldie 1990a). In order to avoid this kind of circularity various attempts have been made to develop an objective technique for aging, one that is free from any need for a prior knowledge of growth patterns. In a recent paper, Fenton et al. (1991) claimed that an aging technique based on ^{210}Pb : ^{226}Ra disequilibrium yields ages for orange roughy, *Hoplostethus atlanticus*, ranging from 77 to 149 years for fish of 38 to 40 cm standard length. The method of Fenton et al. (1991) essentially follows the ^{210}Pb : ^{226}Ra disequilibrium method used by Bennett et al. (1982) for *Sebastes diploproa*, Campana et al. (1990) for *Sebastes mentella*, and Fenton et al. (1990) for *Macruronus novaezelandiae*. This method utilizes the decay of ^{226}Ra through the noble gas ^{222}Rn to ^{210}Pb, and claims that the progressive approach to secular equilibrium of the ^{210}Pb : ^{226}Ra ratio in the fish otolith, from its initial disequilibrium, provides a means of calculating age.

For radiometric ages calculated from ^{210}Pb : ^{226}Ra disequilibria to be valid, some assumptions must be met. These include:

(*i*) ^{226}Ra and ^{210}Pb (and any intermediate products) must be taken up into the otolith at the time of deposition of otolith material at a rate that is always a constant ratio to the rate of mass increase of the otolith.

(*ii*) After formation of otolith material there must be no losses or gains, other than by radioactive decay or ingrowth, of ^{226}Ra and ^{210}Pb or any intermediate product. Further, for the four teleost studies examined here (Bennett et al. 1982; Campana et al. 1990; Fenton et al. 1990, 1991), this closure assumption must extend down to ^{210}Po, since all these studies determine the amount of ^{210}Pb by measuring ^{210}Po and assume that it is in secular equilibrium with ^{210}Pb.

(*iii*) For secular equilibrium to occur, the decay constant for the parent radionuclide must be substantially lower than the decay constant for the daughter, and the parent decay activity must not decrease appreciably during several half-lives of the daughter. These conditions are met for the ^{226}Ra : ^{210}Pb pair.

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We have three principal concerns about the ^{210}Pb : ^{226}Ra disequilibrium method when applied to otoliths. These are:

(1) It is probable that ^{222}Rn diffuses out of the otolith, which is contrary to assumption (*i*) above.

(2) No authors have quantified the sources and sinks of ^{210}Po, ^{210}Pb, or ^{226}Ra or any intermediate products.

(3) The estimated radiometric ages depend on an assumed otolith mass growth-in-time model that is typically constructed using fish "ages" produced by some other aging method, thereby placing the radionuclide disequilibrium aging method in danger of being tautological. We now examine these concerns in more detail.

^{222}Rn Emanation

The decay series from ^{226}Ra to ^{210}Pb passes through the noble gas ^{222}Rn, which has a half-life of 3.825 days (Cochran 1982) and thus has the potential to emanate out of the otolith should the structure of the otolith permit. Bennett et al. (1982) and Fenton et al. (1990, 1991) acknowledge this possibility of emanation as a potential problem.

Bennett et al. (1982) dealt with the radon closure assumption by writing: "Loss of the noble gas ^{222}Rn or other intermediate members of the decay chain between ^{226}Ra and ^{210}Pb from these aragonite otoliths is believed to be negligible, since ^{226}Ra and ^{210}Pb distributions measured in aragonitic corals by Dodge and Thomson (1974) are not compatible with a significant loss of ^{222}Rn or other nuclides in the decay chain." Dodge and Thomson (1974) postulated ages for two Caribbean corals based on periodic bands in the corals and confirmed their postulated ages by measurements of ^{210}Pb in the banded structures. Their confirmation consisted of the regression of the logarithm of ^{210}Pb activity against their postulated age, and from the least squares fitted line they derived an estimate of the half-life of ^{210}Pb of 20.5 years. They cited the nearness of this estimate to the accepted half-life of ^{210}Pb of 22.3 yr (Cochran 1982) as support for their postulated ages. In adjusting their measurements of ^{210}Pb in the banded structures for ingrowth of ^{210}Pb from ^{226}Ra decay, Dodge and Thomson (1974) arrived at 6 dpm/100kg as the activity of ^{226}Ra in their corals. To obtain this figure they assumed that all decaying ^{226}Ra converted into ^{210}Pb within the coral, and justified this assumption by claiming that it "appears to be valid for other natural series nuclides over long time periods", citing Thurber et al. (1965) although Thurber et al. (1965) did not consider the ^{238}U series below ^{226}Ra.

We will now show that the Dodge and Thomson (1974) data are in fact consistent with the loss of ^{222}Rn from coral and thus cannot be used as evidence for nonemanation of ^{222}Rn. Using the data of Table 1 and Figure 2 of Dodge and Thomson (1974), we duplicated their calculations and confirmed their estimate of 20.5 years for the half-life of ^{210}Pb when the activity of ^{226}Ra is assumed to be 6 dpm/100kg. If, as an alternative hypothesis, it is assumed that 25% of decaying ^{226}Ra emanates from the coral as ^{222}Rn and does not ingrow to ^{210}Pb within the coral, then the net activity of ^{226}Ra available for ingrowth is 4.5 dpm/100kg, not 6 dpm/100kg. Using this alternative figure we get an estimated ^{210}Pb half-life of 22.5 yr, closer to the accepted value of 22.3 yr than the 20.5 yr estimate obtained, assuming no ^{222}Rn losses. Therefore, contrary to the assertion of Bennett et al. (1982), the Dodge and Thomson (1974) data are compatible with a substantial (25%) loss of ^{222}Rn by emanation from corals.

Fenton et al. (1990, 1991) supported their assumption of no ^{222}Rn emanation from otoliths "on the observations of numerous authors (reviewed by Veeh and Burnett 1982) that ^{226}Ra and ^{210}Pb distributions in modern aragonite corals are not compatible with any significant loss of ^{222}Rn, or any other nuclides, in the decay chain between ^{226}Ra and ^{210}Pb" (Fenton et al. 1990). The text of the Veeh and Burnett (1982) review dealt with the ^{238}U series down to ^{230}Th and thus made no mention of radon. However, among the papers cited by Veeh and Burnett (1982) in their discussion of coral, two (Veeh and Green 1977; Ku 1976) did discuss the role of ^{210}Pb in methods for aging corals. Veeh and Green (1977) mentioned, without comment, Dodge and Thomson (1974), which we have discussed above, and Moore and Krishnaswami (1972, 1974), who assumed without discussion that there was closure between ^{226}Ra and ^{210}Pb. Ku (1976) cited Dodge and Thomson (1974) without comment, and cited Moore and Krishnaswami (1972) and Moore et al. (1973) commenting that in these papers "Strong deviations from the ideal $^{210}Pb/^{226}Ra$ decay curve were noted in samples showing relatively smooth $^{228}Ra/^{226}Ra$ decrease ..." Therefore, neither Veeh and Burnett (1982) nor any of the papers on coral reviewed by Veeh and Burnett (1982) offers any evidence for the assumption of closure between ^{226}Ra and ^{210}Pb.

The only evidence we could find for the assumption of ^{222}Rn closure was three levels down the citation tree started by Veeh and Burnett (1982): they cited Ku (1976), who cited Moore et al. (1973), who said "We conducted several ^{222}Rn diffusion experiments and could detect no loss, but the experimental conditions may not have approximated the interior of a coral head." Unfortunately, for this experiment neither experimental details or specific results were given by Moore et al. (1973). Therefore, we are at a loss to explain why Fenton et al. (1990) would cite Veeh and Burnett (1982) to support their assumption of no ^{222}Rn emanation. Campana et al. (1990) did not discuss the radon closure assumption.

Does the structure of otolith material permit the loss of radon? Morawska and Phillips (1993) examined the dependence of ^{222}Rn emanation upon the internal structures of the materials in which it is embedded and on the distribution of the parent ^{226}Ra within those materials. They considered two physical processes: firstly, the migration of ^{222}Rn atoms within solid material as a result of recoil energy gained upon the decay of the parent ^{226}Ra; and secondly, diffusion in the event that a ^{222}Rn atom reach and remain in a free space in the material (pore, micropore, or crack). In discussing the recoil mechanism, Morawska and Phillips (1993) cite recoil ranges for ^{222}Rn of 65 μm in air, 50 μm in water, 30 nm in gypsum, 36 nm in glass, and an average of 40 nm in rocks. We would expect the recoil distance of ^{222}Rn in otolith material to be similar to that of rocks, glass, and gypsum. Morawska and Phillips suggest that, should a ^{222}Rn atom exhaust its recoil energy in a free space, then its diffusion length would be 20–200 cm in dry material (such as air) and 2–3 cm in water-filled pores.

In their discussion of migration models, Morawska and Phillips (1993) make the following point: "The conclusion to be drawn is that an investigation of the radon emanation properties of a dispersed material should be preceded by a microscopic study of the physical properties of the material. Lack of knowledge of the intrinsic particle properties of

the material may lead to an inability to understand radon emanation data." Otoliths consist of aggregations of very small crystals with interstitial lamellar spaces. Scanning electron microscope studies of interactions between crystal ultrastructure and microincrement layers in the otoliths of the teleosts *Macruronus novaezelandiae* and *Hoplostethus atlanticus* (Gauldie and Nelson 1988) and *Beryx splendens* (Gauldie and Nelson 1990) determined that the otoliths were composed of twinned aragonite crystals laid down as a series of protein-rich and protein-deficient bands with bundles of crystals orthogonal to the band orientation. Bands were typically 1-3μm wide and within the bands there were repeating stacked layers of orientated crystals that were 100–200nm wide and 1 to several micrometers long. Similar sized otolith crystals have been reported by Degens et al. (1969), Gauldie (1990b), and Lecomte-Finiger (1992) for otoliths of other teleost species. The presence of interstitial spaces between individual crystals is shown by the presence of non calcium-binding dyes and stains within the otolith (Gauldie et al. 1990). The nanometer sizes of the crystals and the presence of interstitial spaces means that the escape of ^{222}Rn from otolith material by recoil and then by diffusion is both possible and highly likely.

The loss of ^{222}Rn from otoliths, and thus less ingrowth of ^{210}Pb, would result in falsely low ages from the ^{210}Pb : ^{226}Ra disequilibrium method.

Sources and Sinks of ^{210}Po, ^{210}Pb, and ^{226}Ra

Studies of ^{226}Ra–^{222}Rn–^{210}Pb–^{210}Po systematics in sea water, particularly near the ocean floor, show a dynamic chemistry in which ^{210}Pb is recycled and scavenged from both the sediment and water column into both dissolved and particulate phases (Nozaki 1986; Chung 1987). Direct analyses of ^{210}Pb in the food chain have shown an increase in ^{210}Pb up the food chain leading to ^{210}Pb levels in the liver of 12 kg *Thunnus thynnus* of 0.044–0.067 dpm·g^{-1} (Heyraud and Cherry 1979). The levels of ^{210}Pb reported by Heyraud and Cherry (1979) were of the same magnitude as the ^{210}Pb levels in the otoliths of *Hoplostethus atlanticus* reported by Fenton et al. (1991). The levels of ^{210}Pb in the otolith samples of Bennett et al. (1982), Campana et al. (1990), and Fenton et al. (1990, 1991) were not measured directly but rather were assayed for ^{210}Pb via its alpha-emitting grand-daughter ^{210}Po (138-day half-life). ^{210}Po is even more concentrated than ^{210}Pb in the tissues of *Thunnus thynnus* (Heyraud and Cherry 1979). The high levels of ^{210}Pb and ^{210}Po were due to concentration along the food chain, and the same effect probably occurs in other top-level predator fishes. Fishes generally contain a considerable amount of lead (e.g., Patterson and Settle 1977), some of which finds its way into the otolith (Grady et al. 1989). The similar concentrations of ^{210}Pb in *Thunnus thynnus* tissue and *Hoplostethus atlanticus* otoliths raises questions about the relative sources of ^{210}Pb in the otolith: how much is allogenic (coming from outside the otolith) and how much is authigenic (coming from the decay of ^{226}Ra within the otolith)? The large samples required for the assay of ^{210}Pb via the ^{210}Po technique mean that it is not possible to check ^{210}Pb levels or ^{210}Pb : ^{226}Ra activity ratios for different sections of otoliths. Without control of the sources of ^{210}Pb (or ^{210}Po) it is difficult to assess the magnitude of allogenic ^{210}Pb or ^{210}Po entering the otolith, either at formation or diffusing later into the otolith through the pore structure, and thus to assess potential errors in age estimations such contaminants introduce. It is usually assumed (e.g., Fenton et al. 1990) that allogenic ^{210}Pb is greatly exceeded by the uptake of ^{226}Ra, and thus allogenic ^{210}Pb and ^{210}Po can be ignored in subsequent calculations.

The assumption that both ^{226}Ra and ^{210}Pb are taken into the otolith at a rate that is in a constant ratio to the rate of mass increase of the otolith is known to not always be true in fishes. Fenton et al. (1990) demonstrated that in *Macruronus novaezelandiae* that the uptake of ^{226}Ra into the otolith decreased as otolith mass increased. The decrease shown by Fenton et al. (1990) was to 10% of initial concentrations. Data presented in Fenton et al. (1990) indicated that the uptake of ^{210}Pb in *Macruronus novaezelandiae* remained fairly constant. This had the effect of boosting the ^{210}Pb : ^{226}Ra uptake activity ratio as otolith mass increased. For radiometric aging this would result in falsely high ages. Because of the decrease in the uptake of ^{226}Ra as otolith mass increases, Fenton et al. (1990) concluded that *Macruronus novaezelandiae* could not be aged by radiometric means. Bennett et al. (1982) tabulated measured ^{226}Ra specific activities for *Sebastes diploproa* ranging from 0.033 to 0.054 dpm·g^{-1} for their four samples and concluded that "specific activities are probably the same within the range of analytical uncertainties." Campana et al. (1990) found that for *Sebastes mentella* there were "no significant differences in ^{226}Ra concentration" between whole otolith samples and core samples. For their calculation, Campana et al. (1990) used a ^{226}Ra specific activity of 0.033±0.002 dpm·g^{-1}, where the error quoted is plus or minus one standard deviation. For *Hoplostethus atlanticus*, Fenton et al. (1991) reported a trend in ^{226}Ra specific activity with their "younger" (i.e., smaller otolith mass) samples having a mean specific activity of 0.0522±0.0036 dpm·g^{-1} and the "older" group (i.e., higher otolith mass) having a ^{226}Ra mean specific activity of 0.0625±0.0030 dpm·g^{-1}. The error quoted is plus or minus one standard deviation. Fenton et al. (1991) used these groupings to calculate their ^{210}Pb : ^{226}Ra ratios.

Clearly, the constancy of the ^{210}Pb : ^{226}Ra uptake ratio at otolith formation must be checked for every application of the ^{210}Pb : ^{226}Ra disequilibrium aging method. It is fairly easy to detect any change in ^{226}Ra uptake because, as ^{226}Ra decays very slowly (4% in each 100 years), one has simply to examine the ^{226}Ra specific activities for samples of otoliths having different masses. Checking ^{210}Pb uptake for otoliths of different masses is more difficult because of the more rapid decay and ingrowth of ^{210}Pb from ^{226}Ra. To check ^{210}Pb uptake requires subsampling of material from within otoliths. None of the four teleost radiometric aging studies published (Bennett et al. 1982; Campana et al. 1990; and Fenton et al. 1990, 1991) reported checking ^{210}Pb uptake as a function of otolith mass, and hence none of the studies verified the assumption of constancy in the ^{210}Pb : ^{226}Ra uptake ratio. The coring method of Campana et al. (1990) will, however, be protected from violation of the constancy assumption (unless the ^{210}Pb : ^{226}Ra ratio fluctuates widely in a very short time) because they restricted their material to cores formed over a small portion of the teleost's life.

The use of a chemical technique that relies on quantification of concentrations also requires the quantification of errors resulting from the sources and sinks of the elements involved. This requirement is all the more stringent when

an inert gaseous intermediate is involved. It is most unsatisfactory to appeal to general studies that have not in any way quantified either ^{222}Rn loss or ^{210}Pb uptake in any carbonate, let alone in otoliths.

The description of the disequilibrium method used by Bennett et al. (1982), Campana et al. (1990), and Fenton et al. (1990, 1991) as the ^{210}Pb : ^{226}Ra method is somewhat of a misnomer, as it is ^{210}Po, the alpha-emitting daughter of ^{210}Pb, that was actually measured in these studies. ^{210}Po is a common contaminant in fishes. Hoffman et al. (1974) wrote: "Polonium-210 is an alpha emitter contributing major portions of the natural burden of ionizing radiation to many marine organisms", and Heyraud and Cherry (1979) provide an extensive account of ^{210}Po distribution in the tissues of a number of fishes. Fenton et al. (1991) also wrote "... extensive pretreatment is necessary to ensure the removal of ... any associated allogenic ^{210}Po", and carefully detail their decontamination procedure. None of the papers that use this ^{210}Po/^{210}Pb : ^{226}Ra disequilibrium offer any calculations on the expected ratio of ^{210}Po taken up by the otolith from the fluid to ^{210}Po resulting from authigenic ^{222}Rn.

Calculation of Ages

The estimation of age using a disequilibrium method requires that the decay constant for the parent radionuclide (in the current case λ_{Ra}) be substantially lower than the decay constant of the daughter (in the current case λ_{Pb}), and that the parent decay activity not decrease measurably during many daughter half-lives. In this situation the daughter activity increases along a simple exponential growth curve dependent only on the daughter's decay constant until, at a time much greater than the daughter's half-life, the decay activities of both the daughter and the parent are equal, and secular equilibrium is achieved. Provided the disequilibrium at the time of material formation is known, then the disequilibrium measured at any later time before secular equilibrium can be used as a clock running from the time of material formation. For a fuller discussion, see chapter 21 of Faure (1986).

In an object like an otolith, where the material is not all laid down at one time, it is necessary to model the material accretion in time in order to use the disequilibrium clock. Since $\lambda_{Ra} << \lambda_{Pb}$ it can be shown that the total quantity of ^{210}Pb in a whole otolith of age T is

$$(1) \quad S_{Pb}^{T} = \int_0^T G(t) A_{Ra}^0 (1 - e^{-\lambda_{Pb} t}) dt + \int_0^T G(t) A_{Pb}^0 e^{-\lambda_{Pb} t} dt$$

where A_{Ra}^0 and A_{Pb}^0 are the specific activities of ^{226}Ra and ^{210}Pb at the time that the material is first accreted and are assumed to be invariant in time, and $G(t)$ is the mass growth rate of the otolith as a function of time, t (see Ivanovich 1982, p14ff, for an explanation of the construction of this equation). For an otolith accreting until age T, at which time it has mass M_T, define $\bar{A}_{Pb}^T$, the mean ^{210}Pb specific activity of the whole otolith aged T, as

$$(2) \quad \bar{A}_{Pb}^T = \frac{S_{Pb}^T}{M_T}$$

Hence, A, the ^{210}Pb : ^{226}Ra activity ratio at time T can be written as

$$(3) \quad A = \frac{\bar{A}_{Pb}^T}{A_{Ra}^0 e^{-\lambda_{Ra} T}}$$

Substituting [2] and [3] into [1] and defining R, the ^{210}Pb : ^{226}Ra activity ratio as the material is accreted to the otolith as

$$(4) \quad R = \frac{A_{Pb}^0}{A_{Ra}^0}$$

yields

$$(5) \quad A e^{-\lambda_{Ra} T} = \frac{1}{M_T} \int_0^T G(t)(1 - e^{-\lambda_{Pb} t}) dt + \frac{R}{M_T} \int_0^T G(t) e^{-\lambda_{Pb} t} dt$$

Equation [5] can be solved for T, the life span of the fish. For all but the simple linear growth model, the solution for T is dependent on the model of otolith accretion in time that is adopted. It is usual to make adjustments to the activity ratio, A, to allow for the time between the collection of the otoliths and the radiometric analysis. The solution of [5] is generally by an iterative numerical method such as Newton's method. Solutions can arise that are outside the window of physical possibility, such as ages less than the time required for the activity ratio A to reach its observed value. Provided that the assumption of a constant ^{210}Pb : ^{226}Ra uptake ratio holds, the absolute lower limit of age can be determined by considering the case where all of the otolith mass is deemed to be laid down at the start of life. In this case

$$(6) \quad A e^{-\lambda_{Ra} t} = (1 - e^{-\lambda_{Pb} t}) + R e^{-\lambda_{Pb} t}$$

T, the age produced as a solution to equation [5], must be greater than t, the solution to equation [6]. Solutions outside the window of physical possibility might indicate that an inappropriate otolith accretion model has been adopted.

Otolith Mass Growth in Time, $G(t)$

To obtain sufficient decay counts in a reasonable time the ^{226}Ra : ^{226}Pb disequilibrium method requires at least a gram of material. Bennett et al. (1982) and Fenton et al. (1990, 1991) pooled several whole otoliths, thought to be of similar age, to obtain this weight. Campana et al. (1990) pooled otolith cores assumed to be of similar age and assumed to contain five years of growth. As otolith material is laid down over the life of the fish, the use of whole otoliths or cores thought to form over several years requires a knowledge of otolith mass growth in time to extrapolate from the mean ^{210}Pb : ^{226}Ra activity ratio of the sample to the age of the sample.

Campana et al. (1990) assumed linear growth in mass over time for their cores. This would seem a reasonable procedure, since linear approximations often hold over short periods of time. Further, even if growth were markedly nonlinear in otolith cores, provided all of each core is accreted in the short period ascribed to core formation, any errors introduced should be less than (±) the period allowed for core formation. (The extremes are determined by considering all the core being accreted instantaneously at either end of the period). Bennett et al. (1982) and Fenton et al. (1990, 1991) used linear growth in mass as either one straight line over the whole life of the fish or a "broken stick" line that

TABLE 1. Physical and radiometric data for otolith samples of *Hoplostethus atlanticus* (Extracted from Table 2 of Fenton et al. 1991.) The errors are expressed at the ± 1 standard deviation level.

Sample number	Mean fish standard length (cm)	Mean otolith mass (mg)	^{210}Pb : ^{226}Ra activity
LH1240	37.30	175.1	0.499±0.065
LH748	39.20	270.6	0.867±0.085
LH749	40.40	367.5	0.806±0.069

had one slope before some maturity point and a second, lower slope after maturity.

The selection of an appropriate otolith growth-in-mass model requires some a priori knowledge of the mass growth in the otolith of the particular species. Such information is difficult to find, even for short periods of time. The validity of the age estimate is crucially dependent on the selection of an appropriate model. So what are appropriate models? It is known that otolith growth can be affected by depth (Wilson 1985) and body weight (Wilson and Larkin 1982), and that there are regional variations for some species (Smith 1992). Bennett et al. (1982) derived their models from whole otolith and section aging techniques based on whole and sectioned otoliths: the very methods whose validity they were trying to test using the ^{210}Pb : ^{226}Ra disequilibrium technique. The danger of circularity is obvious. Fenton et al. (1991) commented: "Reliable values of the mass growth rates of otoliths throughout a species life span are difficult to find in the literature. Bennett et al. (1982), in their radiometric aging study of *Sebastes diploproa*, estimated that growth slowed to 30% of its initial rate beyond an age of 14 yr. We conclude that the ages computed using a single growth rate throughout the life span are not appropriate for orange roughy beyond maturity. A slower growth for the otolith beyond this age is inferred ... For the purposes of the model, maturity was set at 32 years ... A growth rate (G2) after maturity of 45% of the pre-maturity value (1.77 mg yr^{-1}) was also assumed. The selection of G2 is, however, arbitrary, and it should be stressed that the validity of the value cannot be independently confirmed from our data."

Age Estimates Depend on an Otolith Mass Growth-in-Time Model

With the exception of simple linear growth, the age estimates derived from the solution of equation [5] depend upon the otolith mass growth-in-time model adopted. Consider first the case of simple linear growth in time, i.e., $G(t) = c$, where c is a constant. $G(t)$, being a constant, can be removed outside the integrals. Since otolith accretion is linear, $M_T = cT$. Substituting cT for M_T in equation [5] confirms that estimated ages obtained from the solution of equation [5] are independent of c, the rate of otolith accretion.

Examination of equation [5] shows that for all other models, including the "broken stick" model used by Bennett et al. (1982) and Fenton et al. (1991), the ages determined from the solution of equation [5] depend both on the model and on the rate of accretion. This is not a new finding. In a review of the use of the disequilibrium between the radionuclides ^{228}Th and ^{228}Ra for the aging of a deep-sea coral and a deep-sea vesicomyid clam, Cochran (1982) showed that different mass growth-in-time assumptions lead to order-of-magnitude differences in ages assigned from the same ^{228}Th : ^{228}Ra activity ratios. We can demonstrate a similar phenomenon for the ^{210}Pb : ^{226}Ra data reported by Fenton et al. (1991) for *Hoplostethus atlanticus* if we use the same growth model as Fenton et al. (1991) but vary the rate of otolith mass increase. Fenton et al. (1991) modeled the mass growth in time of the otoliths of *Hoplostethus atlanticus* assuming a linear increase in mass with time. They presented two models: one where the same linear otolith growth rate is used for the entire life of the fish; and another (a "broken stick" model) where one linear growth rate, G_1, holds before some supposed maturity age t_m, and a second linear growth rate, G_2, holds after maturity. The "broken stick" model was first presented by Bennett et al. (1982). Note that G_1, G_2 and t_m, together with the mean otolith mass M_T, are fully sufficient to ascribe an age to the fish without any recourse to radiometric methods. The assignment of age T can be made from the equation

$$(7)\quad M_T = G_1 t_m + G_2(T - t_m)$$

If we adopt the "broken stick" growth model, then equation [5], which gives the radiometric estimate of age, simplifies to

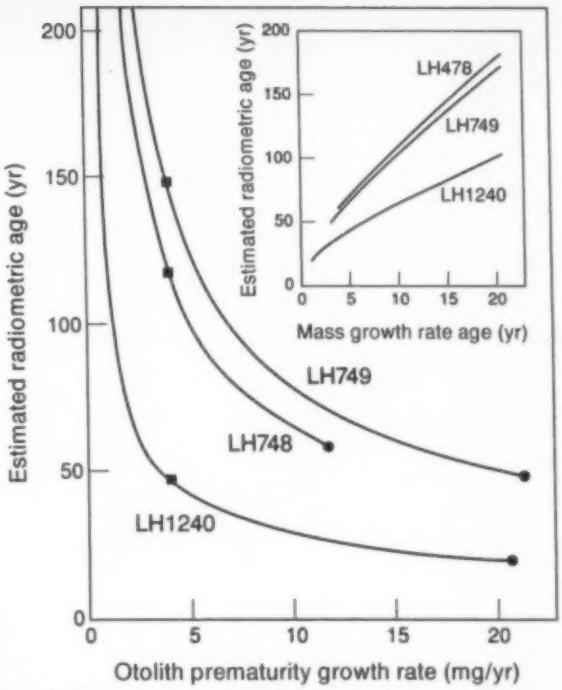

FIG. 1. The main plot shows estimated radiometric age calculated from equation [8] as a function of otolith prematurity growth rate, G_1, for the *Hoplostethus atlanticus* of Table 1. The solid squares indicate the estimates of radiometric age from Fenton et al. (1991) and the solid circles show the minimum admissible values of radiometric age, given that all the assumptions of the radiometric method are met. The insert shows the almost linear dependence of estimated radiometric age on the otolith mass growth rate age derived from equation [7] for the *Hoplostethus atlanticus* of Table 1.

$$(8)\quad Ae^{-\lambda_{Ra}T} = \frac{t_m G_1}{M_T}\left[1-(1-R)\left\{\frac{1-e^{-\lambda_{Pb}t_m}}{\lambda_{Pb}t_m}\right\}e^{-\lambda_{Pb}(T-t_m)}\right] + \frac{(T-t_m)G_2}{M_T}\left[1-(1-R)\left\{\frac{1-e^{-\lambda_{Pb}(T-t_m)}}{\lambda_{Pb}(T-t_m)}\right\}\right]$$

To illustrate the dependence of the radiometric age estimates on the otolith mass growth-in-time rate adopted, we consider three samples (out of 12) reported by Fenton et al. (1991). Some of the sample details reported by Fenton et al. (1991) are shown in Table 1. Fenton et al. (1991) set G_1 at 3.94 mg·yr^{-1}, G_2 at 45% of G_1, t_m at 32 yr, and R at 0.05. λ_{Pb} and λ_{Ra} are physical constants and are 0.03114 yr^{-1} and 0.00043 yr^{-1}, respectively. Suppose we adopt the "broken stick" model for otolith mass growth in time, and vary G_1 and G_2 such that G_2 is always 45% of G_1, as in Fenton et al. (1991). Further, we also require that a fixed otolith mass is accumulated before the maturity age t_m is reached, and we adopt the same mass as used by Fenton et al. (1991), i.e., 126 mg. We then use equation [8] to plot on Fig. 1 the estimated radiometric age against otolith mass growth-in-time rate for the otoliths of Table 1.

The dependence of estimated radiometric age on otolith mass growth in time is clearly shown in Fig. 1, with faster mass growth rates leading to lower radiometric ages. Ages that are less than half those ascribed by Fenton et al. (1991) are sustainable from the same ^{210}Pb : ^{226}Ra activity ratios. Interestingly, ages ascribed under the simple mass growth model of equation [7] are almost linearly correlated with the radiometric ages obtained using equation [8] and the "broken stick" model with the conditions we specified, clearly illustrating the circularity of the method [Fig. 1].

The Use of Otolith Cores

Campana et al. (1990) overcame many of the difficulties that arise from the use of entire otoliths by forming their 1-g samples from between 21 and 28 cores cut from around otolith nuclei. These cores each approximated the linear dimensions of the sagittal otolith of 5-year-old *Sebastes mentella*. The use of cores makes the adoption of a simple linear growth model much more plausible. The adoption of a simple linear model means that the radiometric ages are not dependent on an assumed accretion rate. Further, the maximum error introduced by the selection of an inappropriate model is ±5 yr, provided that the aging method used to determine the core size is correct, and provided that all of the core was accreted within the first five years. Formation of samples for radiometric analysis from cores also reduces the requirement for constancy of the ^{210}Pb : ^{226}Ra uptake ratio from the whole life of the fish to the time period represented by the otolith core. The problems of ^{222}Rn emanation and allogenic contamination are not ameliorated.

The use of otolith cores can be compromised by the coring process. Campana et al. (1990) used a rectangular parallelepiped (i.e., box-shaped) core. If, however, the otolith is accreted as an ellipsoid rather than a rectangular parallelepiped, then only 52% of the material in the box core will be accreted in the first five years, the balance being accreted later. This would have the effect of reducing the ^{210}Pb : ^{226}Ra activity levels of the cores and thus yielding younger than actual radiometric ages. It also reintroduces problems with the assumption of constancy of the ^{210}Pb : ^{226}Ra uptake ratio.

Effects of Assumption Failures on Estimated Radiometric Ages

If ^{222}Rn is lost from otoliths, but the age estimation using equation [5] is carried out on the assumption of no ^{222}Rn emanation, then the estimated ages will be too low. For instance, using the simple constant linear otolith growth rate model, a 16% loss of ^{222}Rn from the otolith material would boost the Fenton et al. (1991) age estimate for *Hoplostethus atlanticus* sample LH1240 from 46.7 to 62 yr, and for sample LH749 from 156 to 469 yr. Similar results hold for otolith growth models other than simple linear growth.

Similarly, if the ratio of allogenic ^{210}Pb to allogenic ^{226}Ra increased during the formation of the otolith, then ages produced by the solution of equation [5] will be too high. Fenton et al. (1990), working on *Macruronus novaezelandiae*, demonstrated that the uptake of ^{226}Ra into the otolith decreased as otolith mass increased. The decrease shown by Fenton et al. (1990) was to 10% of the initial concentration. Data presented in Fenton et al. (1990) indicated that the uptake of ^{210}Pb in the otolith of *Macruronus novaezelandiae* remained fairly constant through otolith accretion. The net effect in the otolith is a boosting of the ^{210}Pb : ^{226}Ra ratio. This effect can be modeled into the equations used for radiometric age estimation. In the event of a decline in ^{226}Ra uptake to k% of its initial value, then equation [5] can be modified to

$$(9)\quad Ae^{-\lambda_{Ra}T} = \frac{1}{M_T}\int_0^T G(t)(1-e^{-\lambda_{Pb}t})\left(1-\frac{(1-k)tG(t)}{100M_T}\right)dt + \frac{R}{M_T}\int_0^T G(t)e^{-\lambda_{Pb}t}dt$$

Equation [9] can be integrated and numerically solved for T. (With the assumption of simple constant linear otolith growth, the terms M_T and $G(t)$ disappear, as before.) Assuming that the uptake of ^{226}Ra declines to 10% of its initial value at time T in *Hoplostethus atlanticus* (as Fenton et al. (1990) report for *Macruronus novaezelandiae*) and that there is constant linear otolith growth, then the age estimate for sample LH1240 declines from 46.7 to 41.3 yr, and the age estimate for sample LH749 declines from 156 to 109 yr. Similar results hold for otolith growth models other than constant linear growth.

Conclusions

Both the whole otolith technique used by Bennett et al. (1982) and Fenton et al. (1990, 1991), and the otolith core technique of Campana et al. (1990) are vulnerable to any ^{222}Rn emanation from the otolith. The references cited by Bennett et al. (1982) and Fenton et al. (1990, 1991) to support their assumption of no ^{222}Rn emanation do not, as shown by the review in this paper, support that assumption. The ^{222}Rn emanation models presented by Morawska and Phillips (1993), when considered with current knowledge of otolith microstructure, provide realistic and very plausible mechanisms for ^{222}Rn emanation from otoliths. It may be

possible with a detailed knowledge of the otolith microstructure of each species, or by experimental determination, to estimate and correct for ^{222}Rn emanation. Estimation of ^{222}Rn emanation by modeling is more likely to be feasible for the relatively homogenous and regularly shaped cores used in the Campana et al. (1990) method than for the method using whole otoliths.

Fenton et al. (1990) demonstrated that in *Macruronus novaezelandiae* uptake of ^{226}Ra per gram of otolith accreted decreased as otolith mass increased, but that the uptake of ^{210}Pb remained fairly constant. This had the effect of boosting the ^{210}Pb : ^{226}Ra activity ratio as the otolith mass increased, yielding falsely high ages. For the four teleost species for which ^{210}Pb : ^{226}Ra disequilibrium aging techniques have been published, the essential assumption that there is a constant ^{210}Pb : ^{226}Ra uptake ratio has been shown to be not true for *Macruronus novaezelandiae*, and not reported as tested for *Sebastes diploproa*, *Sebastes mentella* and *Hoplostethus atlanticus*. The *Sebastes mentella* study by Campana et al. (1990) may be protected, by the use of otolith cores, from the problem of variation in uptake rates.

We have demonstrated that the age estimates produced by the ^{210}Pb : ^{226}Ra disequilibrium method are critically dependent on the otolith mass growth-in-time model adopted. For the activity ratios and otolith masses of *Heplostethus atlanticus*, and the otolith mass growth-in-time model as published by Fenton et al. (1991), the use of faster otolith accretion rates yields ages less than half those advanced by Fenton et al. (1991).

The use of otolith cores limits errors introduced by the adoption of an inappropriate otolith mass growth-in-time model. Cores also limit the period for which the assumption of constancy in the ^{210}Pb : ^{226}Ra uptake ratio must hold to the time necessary to accrete the core. For these advantages to accrue, cores must closely approximate the shape of the otolith at the time the core was formed. The use of cores will not ameliorate the problem of ^{222}Rn emanation, but it does bring the disequilibrium method closer to the objective ideal of being independent of the growth rate of the otolith.

We conclude that failure to test or meet essential assumptions, and critical dependence on the otolith mass growth-in-time model adopted, disqualifies current ^{210}Pb : ^{226}Ra disequilibrium techniques as a means of validating teleost ages. Further experimental and theoretical studies of the properties of otoliths might hold promise of providing corrections for ^{222}Rn losses. Similarly, increased effort in understanding the biochemistry and physiology of the otolith will improve our understanding of the sources and sinks of radionuclides. Radionuclide disequilibrium methods hold great promise in aging studies, but that great promise is matched by technical difficulties that have yet to be solved.

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References

Bennett, J.T., G.W. Boehlert, and K.K. Turekian. 1982. Confirmation of longevity in *Sebastes diploproa* (Pisces: Scorpaenidae) from ^{210}Pb/^{226}Ra measurements in otoliths. Mar. Biol. 71: 209–215.

Campana, S.E., K.C.T. Zwanenburg, and J.N. Smith. 1990. ^{210}Pb/^{226}Ra determination of longevity in redfish. Can. J. Fish. Aquat. Sci. 47: 163–165.

Chung, Y. 1987. ^{210}Pb in the western Indian Ocean: distribution, disequilibrium, and partitioning between dissolved and particulate phases. Earth Planet. Sci. Lett. 85: 28–40.

Cochran, J.K. 1982. The use of naturally occurring radionuclides as tracers for biologically related processes in deep-sea sediments. *In* W.G. Ernst and J.G. Morin (eds). The environment of the deep sea. Rubey 2, Prentice-Hall, New Jersey, pp. 56–72.

Degens, E.T., W.G. Deuser, and R.L. Haedrich. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. 2: 105–113.

Dodge, R.E., and J. Thomson. 1974. The natural radiochemical and growth records in contemporary hermatypic corals from the Atlantic and Caribbean. Earth Planet. Sci. Lett. 23: 313–322.

Faure, G. 1986. Principles of isotope geology, 2nd ed. John Wiley and Sons, New York. 589 pp.

Fenton, G.E., D.A. Ritz, and S.A. Short. 1990. ^{210}Pb/^{226}Ra disequilibria in otoliths of blue grenadier, *Macruronus novaezelandiae*; Problems associated with radiometric ageing. Aust. J. Mar. Freshwater Res. 41: 467–473.

Fenton, G.E., S.A. Short, and D.A. Ritz. 1991. Age determination of orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae) using ^{210}Pb : ^{226}Ra disequilibria. Mar. Biol. 109: 197–202.

Gauldie, R.W. 1990a. How often is the von Bertalanffy-type length-at-age curve in fishes related to weight change artefacts interpreted as age rings in otoliths? Comp. Biochem. Physiol. A, 96(4): 451–458.

Gauldie, R.W. 1990b. Phase differences between check ring locations in the orange roughy otolith (*Hoplostethus atlanticus*). Can. J. Fish. Aquat. Sci. 47: 760–765.

Gauldie, R.W., and D.G.A. Nelson. 1988. Aragonite twinning and neuroprotein secretion are the cause of daily growth rings in fish otoliths. Comp. Biochem. Physiol. A, 90(3): 501–509.

Gauldie, R.W., and D.G.A. Nelson. 1990. Interactions between crystal ultrastructure and microincrement layers fish otoliths. Comp. Biochem. Physiol. A, 97(4): 449–459.

Gauldie, R.W., M.N. Davies, G. Coote, and I. Vickridge. 1990. The relationship between organic material and check rings in fish otoliths. Comp. Biochem. Physiol. A, 97: 461–474.

Grady, J.R., A.G. Johnson, and M. Sanders. 1989. Heavy metal content in otoliths of king mackered (*Scombromorus cavalla*) in relation to body length and age. Contrib. Mar. Sci. 31: 17–23.

Heyraud, M., and R.D. Cherry. 1979. Polonium-210 and lead-210 in marine food chains. Mar. Biol. 52: 227–236.

Hoffman, F.L., V.F. Hodge, and T.R. Folsom. 1974. Polonium radioactivity in certain mid-water fish of the eastern temporal Pacific. Health Phys. 26: 65–70.

Ivanovich, M. 1982. The phenomenon of radioactivity. *In* M. Ivanovich and R.S. Harmon (eds), Uranium series disequilibrium: applications to environmental problems. Oxford University Press, New York, pp. 1–32.

Ku, J.-L. 1976. The uranium-series methods of age determination. Annu. Rev. Earth Planet. Sci. 4: 347–379.

Lecomte-Finiger, R. 1992. The crystalline ultrastructure of otoliths of the eel (*A. anguilla* L. 1758). J. Fish Biol. 40: 181–190.

Moore, W.S., and S. Krishnaswami. 1972. Coral growth rates using ^{228}Ra and ^{210}Pb. Earth Planet. Sci. Lett. 15: 187–190.

Moore, W.S., and S. Krishnaswami. 1974. Correlation of X-radiography revealed banding in corals with radiometric growth rates. Proc. Int. Coral Reef Symp., 2nd. 2: 269–276.

Moore, W.S., S. Krishnaswami, and S.G. Bhat. 1973. Radiometric determinations of coral growth rates. Bull. Mar. Sci. 23: 157–176.

Moraswka, L., and C.R. Phillips. 1993. Dependence of the radon emanation coefficient on radium distribution and internal structure of the material. Geochim. Cosmochim. Acta, 57: 1783–1797.

Nozaki, Y. 1986. ^{226}Ra-^{222}Rn-^{210}Pb systematics in seawater near the bottom of the ocean. Earth Planet. Sci. Lett. 80: 36–40.

Patterson, C., and D. Settle. 1977. Comparative distributions of alkalies, alkaline earths and lead among major tissues of the tuna *Thunnus alalunga*. Mar. Biol. 39: 289–295.

Smith, K.M. 1992. Regional differences in otolith morphology of the deep slope red snapper *Etelis carbunculus*. Can. J. Fish. Aquat. Sci. 49: 795–804.

Thurber, D.L., W.S. Broecker, R.L. Blanchard, and H.A. Potratz. 1965. Uranium-series ages of Pacific atoll coral. Science (Washington, D.C.), 149: 55–58.

Veeh, H.H., and W.C. Burnett. 1982. Carbonate and phosphate sediments. *In* M. Ivanovich and R.S. Harmon (eds). Uranium series

disequilibrium: applications to environmental problems. Oxford University Press, New York. pp. 459–480.

VEEH, H.H., AND D.C. GREEN. 1977. Radiometric geochronology of coral reefs. *In* O.A. Jones and R. Endean (eds). Biology and geology of coral reefs, 4: 183–200. Academic Press, New York.

WILSON, K.H., AND P.A. LARKIN. 1982. Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry. Can. J. Fish. Aquat. Sci. 39: 1335–1339.

WILSON, R.R. 1985. Depth-related changes in sagitta morphology in six macrourid fishes of the Pacific and Atlantic oceans. Copeia, 1985: 1011–1017.

The Morphological Basis of Fish Age Estimation Methods Based on the Otolith of *Nemadactylus macropterus*

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Relationships between the anatomy of the endolymphatic sac, sac otoconia, sagitta and astericus of *Nemadactylus macropterus* (Cheilodactylidae: Teleostei) are described and the variation in opaque and hyaline zones, sulcal checks, and daily microincrement widths along different growth axes of the otolith measured. The quantitative morphology of the otolith is used to compare the different age estimation techniques for *N. macropterus* that are based on opaque and hyaline zones, sulcal checks, and daily microincrements. Comparison of the different methods shows that at the present state of knowledge daily microincrements present the most credible age estimation method for *N. macropterus*.

Les rapports entre l'anatomie de la cavité endolymphatique, des otoconies, de la sagitta et de l'astericus de *Nemadactylus macropterus* (Cheilodactylidae: Teleostei) sont décrits et les variations des zones opaques et hyalines, des cercles de croissance du sulcus et de la microprogression quotidienne en longueur selon différents axes de croissance des otolithes, sont mesurées. Les données quantitatives sur la morphologie des otolithes servent à comparer les différentes techniques d'estimation d'âge chez *N. macropterus* qui sont fondées sur l'étude des zones opaques et hyalines, des sillons longitudinaux et des micro-allongements quotidiens. La comparaison des différentes méthodes montre que dans l'état actuel des connaissances, la microprogression quotidienne selon différents axes de longueur constitue la méthode d'estimation de l'âge qui donne les résultats les plus plausibles chez *N. macropterus*.

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Estimation of age in fishes has always been problematical. Over the last hundred years most of the methods of ageing fishes from otoliths have cycled through periods of acceptance and rejection. Methods have been based on length modes (Petersen 1892; Westrheim 1973), periodic structures in scales (Lee 1920; Graham 1929; Kelley 1988), otoliths (Dannevig 1933; Kelly and Barker 1961; Beamish 1979) and sometimes other hard parts of fishes (e.g., spines; McFarlane and Beamish 1987). In the last 10 years there has been a looming conflict between age estimations based on the pattern of checks in the sulcal side of the otolith that usually yield older estimates of age (e.g., Beamish 1979), and estimates of age based on the daily microincrement method (e.g., Ralston and Williams 1988) that usually yield younger ages. These two techniques are fundamentally different in approach; one depends on pattern recognition while the other relies on measurement of the physical dimensions of both the growth axes and the average microincrement widths along those axes.

Some of the difficulties in ageing can be attributed to a fundamental problem in the general dogma of age estimation: that of "validation." There is a widely held belief amongst fish biologists that "validation", "accuracy", and "precision" can be brought together as a scientifically acceptable way to test an age estimation hypothesis. In my opinion, they cannot. It has been demonstrated by Popper (1983, pp. 262–281) that "validation" is based on confirming instances, and that "accuracy" and "precision" are secondary aspects of hypothesis testing. Popper shows that "validation" really means obtaining the anticipated results: all theories, good and bad, give the anticipated result. Consequently, different ageing techniques that may yield up to a four times difference in maximum ages can still legitimately claim to have been "validated." Further difficulties arise from a lack of understanding of the morphological relationships between the various structures (checks, opaque zone, microincrements, etc.) used in estimating age from otoliths. A better procedure would be to establish quantitative, morphological properties of otoliths following Degens et al. (1969) and Dunkelburger et al. (1980) so that various ageing methods can be compared directly with each other in terms of their morphology, not just in terms of frequencies of counts.

In this paper I provide the morphological basis of the structure of both the otolith and the endolymphatic sac in order to compare three different methods of age estimation based on otoliths: (1) integrated daily microincrement widths; (2) whole otolith opaque and hyaline zones; and (3) sulcal checks. The comparisons is made using sagittal otoliths of *Nemadactylus macropterus* (Cheilodactylidae: Teleostei). Specifically, I will describe the morphology of the otolith and the relationships that exist between whole otolith zones, sulcal checks and microincrements, and the physical dimensions of the otolith. I have chosen *N. macropterus* because of the availability in the literature of ages from the whole otolith zone method (Tong and Vooren 1972; Smith 1982) and the method of sulcal checks (Vooren 1977), and because of the importance of age estimation in its management (e.g., Smith 1983; Wankowski et al. 1988).

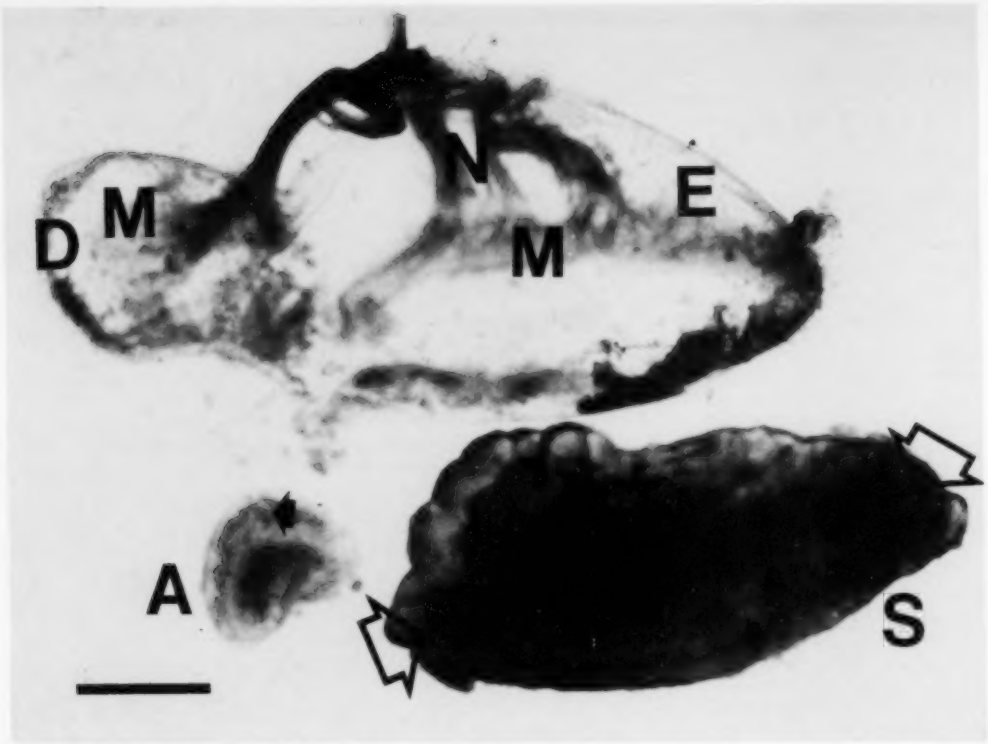


FIG. 1. Dissection of the endolymphatic sac (E) of *Nemadactylus macropterus* shows the nerves (N) that supply the maculae (M) in both the main part of the sac and in the diverticulum (D) that houses the astericus. The sagitta (S) is much larger than the astericus (A) and both show traces of zones (arrows) in this transmitted-light image. Both sagitta and astericus are in their original orientation, dorsal uppermost, anterior to the left. Traces of the sulcus (open arrows) underneath the sagitta can be seen by transmitted light. Scale bar = 1 mm.

Materials and Methods

The nomenclature followed here is *Nemadactylus macropterus* although the same species is referred to in some of the older literature as *Cheilodactylus macropterus* (Ayling and Cox 1982; pp. 241–246). Otoliths were taken from collections made in New Zealand waters between 1967 and 1989. Otoliths were collected at sea and stored dry in paper envelopes. Fish lengths were measured as fork length (FL) to the nearest cm. Dissections of the endolymphatic sac were made from freshly caught specimens. Preparations of otolith and macula for scanning electron microscopy (SEM) have been described in Gauldie et al. (1986). SEM pictures were taken with a Philips 505 SEM. Whole otoliths were photographed with a WILD stereomicroscope, and whole otolith axial distances were measured with a WILD ocular microscope. General methods for preparing otoliths for broken and microincrement sections followed Gauldie (1991). Oxytetracycline was administered at a dose of approximately 1 mg per kg fish weight using veterinary grade dissolved and buffered oxytetracycline.

When examined by either reflected or transmitted light, whole otoliths often show concentric opaque and hyaline zones that form patterns in which the interval between zones (i.e., hyaline to hyaline, or opaque to opaque) decreases more or less monotonically. It is this monotonically decreasing pattern that forms the basis of age estimation using either the opaque and hyaline zone or the check method (May 1965). Quantifying such patterns has proved to be very difficult. Avoiding the uncertainty of the subjective patterns problem has been a major pre-occupation of many studies of ageing from otoliths in which a general consensus of the "right" pattern has been sought (Keir 1960; Kohler 1964; Beamish and McFarlane 1983). The uncertainty of the pattern problem can be alleviated by quantifying the pattern of marks as a series of distances from the nucleus along the growth axes of the otolith. This approach quantifies patterns as the width of the interval between checks as a function of the distance to the distal edge of the otolith from the nucleus. Then cumulative width by distance gives a growth curve for the otolith itself for the particular axial distance involved.

Quantification of otolith zone and check interval patterns by distance provides a quantitative morphological basis for comparison with microincrement width by distance. By casting check and microincrement data in the form of a dependent variable of axial distance, other morphological relationships that depend on axial distance can also be shown. For example, the mass of the otolith is related to its size, so that there is a relationship between otolith mass and axial distance.

TABLE 1. The weight of otoconia relative to the weight of the otolith and the dimensions of the macula of *Nemadactylus macropterus*.

Otolith length (mm)	Otolith weight (mg)	Estimated otoconia weights as % of otolith	Anterior macula (mm)	Posterior macula (mm)	Macula segment ratio
3.00	2.9	0.7	1.00	2.07	0.48
3.35	3.9	0.6	0.98	2.09	0.47
4.25	6.0	0.7	1.23	2.48	0.5
5.00	11.2	0.7	1.42	2.51	0.57
5.85	13.3	0.7	1.82	3.52	0.52
6.05	12.7	0.6	1.84	3.78	0.49
6.50	16.9	0.7	1.88	3.63	0.52
7.05	16.4	0.1	1.94	3.95	0.49
7.10	17.2	0.6	1.92	4.00	0.48
7.25	17.1	0.6	1.84	3.78	0.49
7.30	17.1	0.5	2.04	4.15	0.49
7.35	17.8	0.5	2.30	4.15	0.55
7.50	19.8	0.2	2.43	4.00	0.61
7.70	20.2	0.4	2.23	4.12	0.54
8.15	27.9	0.6	2.55	4.77	0.53
8.60	39.8	0.5	2.48	5.01	0.49

Results

Anatomy of the Endolymphatic Sac

The endolymphatic sac contains the sagitta and astericus otoliths (Fig. 1) and is housed in a complex structure, the auditory capsule, that is formed by the fusion of five bones: the epiotic, pterotic, opisthotic, prootic, and sphenotic (Parker 1895; pp. 92–93). In the Cheilodactylids the skull is deep and the auditory capsule is crowded into a peaked part of the skull above and behind the eyesocket (Gregory 1933; Fig. 136, p. 86).

Dissection of the sac (Fig. 1) shows the smaller anterior astericus and the larger sagitta that each have a separate maculae and nerve supply, but are contained in the same endolymphatic fluid with a constriction in the sac resulting in an anterior diverticulum that houses the astericus. Closer inspection of the sac shows a number of small granules, the otoconia, and separate, well-innervated maculae for both the astericus and the sagitta. Only the sagitta has been used in age estimation in *Nemadactylus macropterus*. Otolith refers to the sagitta unless otherwise stated in the text. Both the sagitta and astericus, which are in their original orientation in Fig. 1, show zones by transmitted light. Traces of the sagittal sulcus, visible in Fig. 1, show a rough correspondence to the size and shape of the macula.

The Macula

The macula is constricted about one third of the length from the posterior end and tends to curve toward the ventral side towards the anterior end (Fig. 2a). Clusters of otoconia are present both on and near the macula (Fig. 2a). Otoconia are of the aragonite crenellated spherule type (Gauldie et al. 1986) that are about 40 μm in diameter and are loosely bound together by fibrous strands (Fig. 2b). Otoconia are large compared with the kinocilia of the macula (Fig. 2b). The kinocilia of the macula are principally of a single type composed of a group of short bundles with one longer protruding cilium (Fig. 2c). Occasionally, a second type of kinocilia with much larger cilia appears on the macula (Fig. 2d). The cell surface between kinocilia is granular and organized into structures of similar dimensions to the kinocilia (Fig. 2c). The kinocilia of the macula do not show any apparent directionality or large-scale organization (Fig. 2d). The relative dimensions of the anterior and posterior segments of the macula are listed in Table 1. The ratio of anterior to posterior segment size does not change appreciably with increasing size (and changing shape) of the otolith.

Otoconia

Dissection of the sac to remove the macula causes disturbance of the otoconia (Fig. 3) that are not tightly bound to the macula or to the sac wall. However, counts of otoconia within the sac can be made from dissections, and estimates of the contribution of the otoconia to the calcium chemistry of the sac can be made. For example, the sac shown in Fig. 3 contained a sagitta weighing 27.9 mg that was 7.55 mm long and 3.42 mm wide from a 37 cm (FL) male fish. An estimate of otoconia numbers, combined with average otoconia size, yields a total weight of 0.19 mg of otoconia (calcium carbonate) material, approximately 0.7% of the weight of the sagitta. If it were assumed that an otolith weighing 27.9 mg was a simple sphere, then a daily increment of 2.5 μm width over half the surface whole sphere would require about 0.07 mg of new otolith material per day. Otolith incrementation is likely to be at a lower rate, but even this conservative estimate shows that the otoconia could provide a reservoir with more than enough calcium carbonate to supply daily microincrementation. Dissected sacs of 16 further otoliths were examined and the otoconial mass in the endolymphatic sac as a percentage of otolith weight was calculated (Table 1). The variation in estimated otoconial weight is compromised by the disturbance due to dissection, but even with such unknown losses there are significant amounts of calcium carbonate contained in the otoconia, which constitute a significant fraction of the weight of the otolith.

Whole Otolith Structure

Whole otoliths of *Nemadactylus macropterus* viewed by transmitted light showed the concentric opaque and hyaline

a
L
A
A
C
b
M
d
c

zone patterns (Fig. 4b) which have been described by Tong and Vooren (1972) and Smith (1982). Viewed by reflected light, the same otoliths showed a concentric surface sculpturing (Fig. 4a) that conforms exactly with the opaque and hyaline zone pattern. Examination of the otolith surface in Fig. 4a showed that the surface sculpturing takes the form of a series of rounded steps separated by narrow depressions. When the otolith was examined sulcus side upwards, little or no evidence of zones was present by either reflected or transmitted light (Figs. 4c, 4d). Examination of the sulcal side by reflected light showed that there is no concentric sculpturing on the sulcal side (apart from the sulcus itself) that could give rise to the impression of opaque and hyaline zones, but the prismatic structure of the otolith was clearly evident.

The opaque and hyaline zones viewed from the antisulcal surface of the otolith in Fig. 4a were not uniform in either width or number in the different growth axes of the otolith. Inspection of the otolith illustrated in Fig. 4a showed that fewer zones lay along the nucleus – ventral growth axis, with more along the nucleus – posterior dorsal growth axis and two to three times as many along the ventral – anterior ramus growth axis. The widths of opaque and hyaline zones of three different otoliths were measured along each of these axes. Cumulative growth curves of otolith material were similar in different otoliths, but growth curves inferred from opaque and hyaline zone cumulative growth patterns in different growth axes would be very different (Fig. 5).

A SEM of the antisulcal surface of the otolith of *Nemadactylus macropterus* (Fig. 6a) confirms that surface sculpturing leads to the observed opaque zone pattern, but also reveals finer scale surface sculpturing. The otolith antisulcal surface shows a range of width in surface steps from very narrow through to the larger features seen by the light microscope. The narrow surface steps (Fig. 6b) are about 3.5 μm wide and are formed on a surface of granular crystals that range from 0.4 to 0.5 μm in diameter. The ventral half of the otolith surface is rougher than the dorsal half with surface steps formed by irregular blocks of crystals (Fig. 6c). The surface steps are about 20 μm wide and are formed from block-like crystals of similar diameter. These SEM studies show variation in the morphology of surface sculpturing in different parts of the otolith surface, as well as the range of widths of surface sculpturing from 20 μm to millimetres.

Opaque and Hyaline Zone Patterns

Consequent to the use of the pattern of opaque and hyaline zones as annual marks, there is an additional use; that of validating the annual periodicity of annual marks by the method of marginal increments, which has been used by both Tong and Vooren (1972) and Smith (1982). The method of marginal increments follows the argument that the narrow hyaline zone observed by reflected light is formed in winter during the period of limited growth, and that the wide opaque zone is formed during the more rapid growth of spring and summer. The austral seasons were defined by Tong and Vooren (1972) as: Spring, September to November; Summer, December to February; Autumn, March to May; and Winter, June to August. Validation of annual periodicity in the pattern can therefore be obtained by sampling the fishery at regular intervals and measuring the increase in the width of the opaque zone (the marginal increment) over the summer. In the usage of Tong and Vooren (1972), the marginal increment was always part of the last opaque zone. The same terminology was followed by Smith (1982; p. 247), quoting Tong and Vooren (1972). In Tong and Vooren (1972; pp. 16–19) the argument is advanced that the opaque zone (reflected light) should increase in width (increasing marginal increment) during the austral spring/summer, and that the opaque zone should cease (no marginal increment) during the formation of the hyaline zone in the austral winter. Marginal increment width data were presented in Tong and Vooren (1972) as the percentage of widths over three age classes (4, 5, 6) by month. Re-analyses of the marginal increment data by months are shown in Figs. 7a and 7b for the age groups 4 and 5, respectively, from Tong and Vooren (1972). Both Figs. 7a and 7b show a *decrease* in marginal increment width over the austral spring/summer. A polynomial (3rd order) curve fitted to the data suggests that the marginal increment width has an apparent annual periodicity with *minimum* widths in the austral spring/summer and *maximum* widths in the austral autumn/winter. Both the linear and polynomial curve fits indicate very weak relationships between marginal increment and season with r^2 values below 0.2.

The data in Smith (1982; p. 247) were not as readily reanalyzed as that of Tong and Vooren (1972), but mean marginal increment width by two-month intervals reported by Smith (1982) (Fig. 7c) *decreased* in the austral spring/summer with a *maximum* at the beginning of spring. A polynomial curve fitted to Smith's data suggests that marginal increment width has an apparent annual periodicity, but with a *minimum* in the late austral summer autumn, and a *maximum* in the late austral spring (Fig. 7c).

Whole Otolith Dimensions

A sample of 76 otoliths taken from fishes ranging in size from 14 to 50 cm (FL) were weighed and measured along four axes: 1) the nucleus–ventral axis; (2) the nucleus–dorsal axis; (3) the nucleus–posterior dorsal axis; and (4) the total length of the otolith. Comparisons of these variables revealed a number of interesting relationships. Otolith total length was linearly related to fish length in both males and females, but showed an increase in scatter with increasing length (Fig. 8a). Otolith weight had a nonlinear relationship to fish weight with apparently different curves for males and females that diverge at about 750 g fish weight from an apparently uniform curve below 750 g (Fig. 8b). Otolith weight showed a nonlinear relationship with fish length with apparently different curves for males and females that diverge at about 35 cm length (Fig. 8c).

The relationship between otolith weight and otolith total length (Fig. 8d) was more complex. Female fishes showed a

FIG. 2. *Nemadactylus macropterus*: (a) SEM of the macula shows the constriction (C) and the curve towards the ventral (V) anterior (A) end. Clusters of otoconia are marked with arrows. Scale bar = 1 mm. (b) Otoconia are bound together by fibrous strands (open narrow). The kinocilia (short arrows) of the macula (M) are very small in comparison with the otoconia. Scale bar = 100 μm. (c) Kinocilia are principally of the type with a group of short cilia (open arrow) and a single long cilium (arrow). The cellular surface between kinocilia is granular (C) and organized into more or less circular areas. Scale bar = 10 μm. (d) Kinocilia have a uniform appearance over the macula surface with occasional kinocilia with much larger cilia (arrow). Scale bar = 100 μm.

FIG. 3. The endolymphatic sac of *Nemadactylus macropterus* shows scattered and clustered otoconia (arrows). Scale bar = 1 mm.

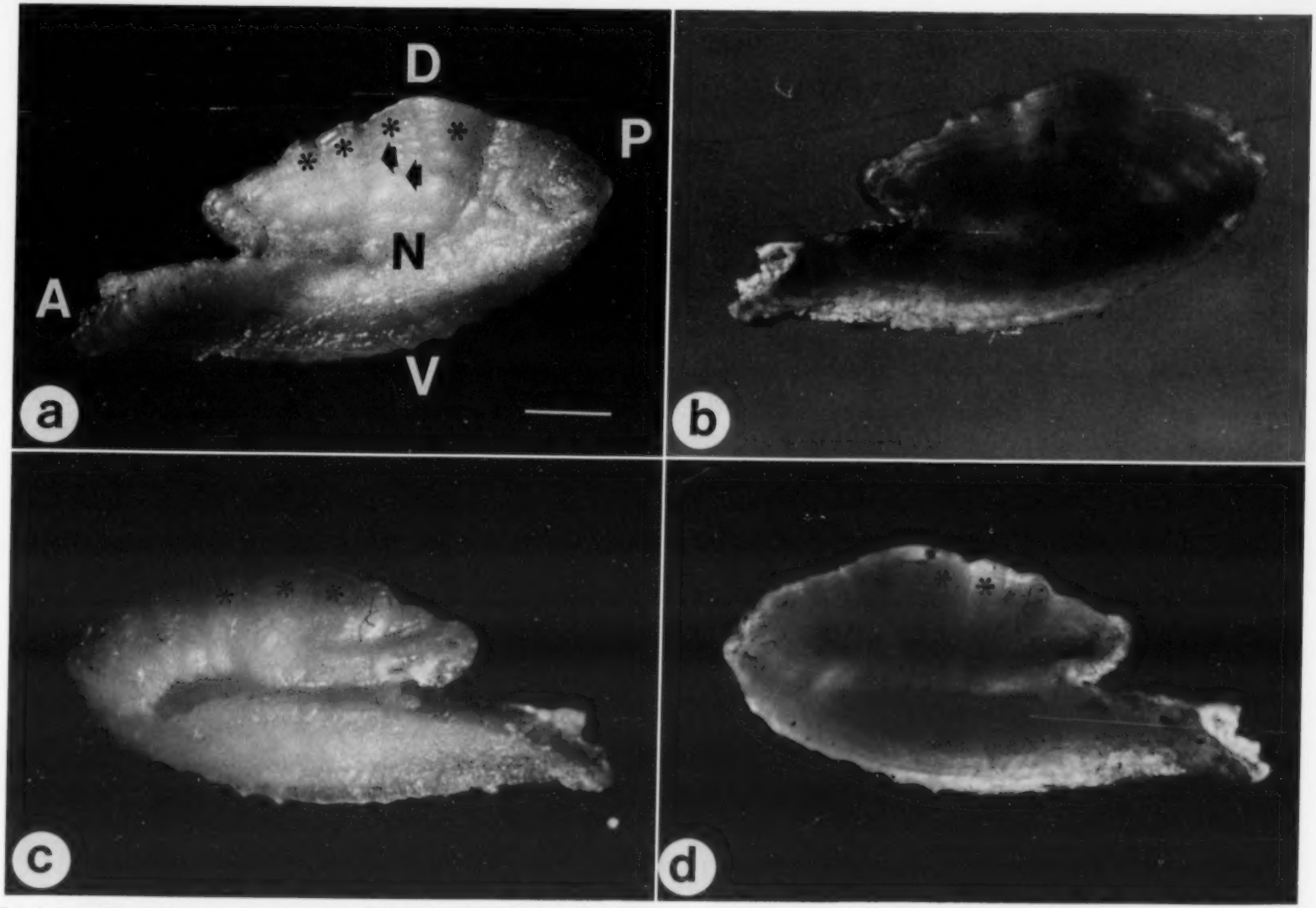


FIG. 4. An otolith of *Nemadactylus macropterus* shown from the antisulcal side by reflected (a) and transmitted (b) light, and from the sulcal side by reflected (c) and transmitted (d) light. The hyaline zones shown by transmitted light (arrows in (b)) correspond to the surface depressions (arrows in a). Dorsal (D) posterior (P) prisms (*) curve as they grow from the nucleus (N) toward the edge of the otolith. Prisms are evident in both reflected (c) and transmitted (d) light images of the sulcal side of the otolith, with weak traces of narrow opaque zones in (d) along the ventral (V) edge. (a) and (b) show that opaque and hyaline zones vary in number between the nucleus to posterior dorsal (N–D), nucleus to ventral (N–V), and the nucleus to anterior ramus (N–A) axes. Scale bar (a) = 1 mm.

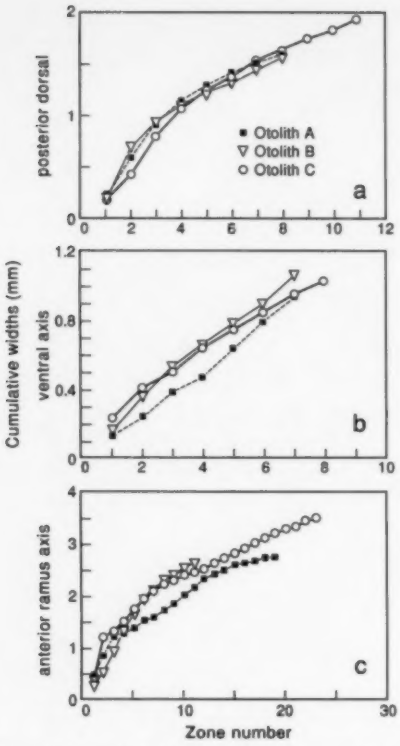


FIG. 5. Cumulative width (mm) of the distances between successive hyaline (by transmitted light) zones shown by successive zone number for three otoliths of *Nemadactylus macropterus* (A, 5.7 mm TL; B, 4.867 mm TL; C, 6.38 mm TL) for the nucleus – posterior dorsal axis (a), the nucleus–ventral axis (b), and the nucleus – anterior ramus axis (c).

nonlinear length–weight curve with increasing scatter with increasing size. Males showed two distinct curves, with six individuals showing almost twice the weight–length ratio as other males. This is very difficult to explain, as the same otoliths do not show any abnormal distributions in the otolith length – fish length and the otolith weight – fish weight curves. The otolith of *Nemadactylus macropterus* has a long anterior rostrum that can be damaged (e.g., Smith (1982) p. 247) but this would result in a decrease in weight, not an increase; nor would the loss of the rostrum be great enough to account for a doubling in weight/length ratios.

Otolith weight showed nonlinear relationships with both the nucleus posterior dorsal otolith axis and the nucleus – ventral growth axis that were similar in males and females (Fig. 8e and f). The shape of the curve in Fig. 8f indicated that otolith weight continued to increase even after the rate of growth of the nucleus–ventral axis became very slow. In both males and females the nucleus ventral axis showed the least increase in length with fish length and the most linear relationship (Fig. 8g and h); the nucleus – posterior dorsal axis showed the greatest increase in length with the least linear relationship, while the nucleus – dorsal axis was intermediate between the other two. The nucleus – posterior dorsal axis had the greatest growth in length with fish length (Fig. 8g and h) and was very similar to the graph of otolith total length plotted against fish length (Fig. 8a).

Broken Sections of Otoliths

Simple dorso-ventral broken surfaces showed that there was no obvious relationship between the internal crystal structure of the otolith and surface sculpturing. The sections in Figs. 9a and 9b showed strong surface sculpturing, but no internal marks corresponding to the surface sculpturing. The section in Fig. 9a shows that although there was radial epitaxial growth of crystal from the nucleus along the dorso-ventral axis, the medial crystals of the sulcal side were much longer (about 2.5 times) than the medial crystals of the antisulcal side. It was evident that in terms of crystal structure the antisulcal and sulcal crystal growth fields have different growth rates.

Broken sections of certain otoliths sometimes showed patches of a characteristically smooth, but still granular, surface which has been described as a spalling surface (Gauldie 1991). Spalling surfaces in broken sections of the otolith of *Nemadactylus macropterus* generally have the fine granular surface (Fig. 9c) typical of spalling lines in other species. However, some otoliths of *N. macropterus* showed a variation in the surface structure of the spalling line. This surface retained the typical granular crystalline surface (Fig. 9d), but showed an unusually coarse plate-like underlying crystal structure.

The plate-like crystal structure of the otolith of *Nemadactylus macropterus* is also unusual in showing periodic structures in broken, unetched sections of the same order of magnitude as daily microincrements. The broken section in Fig. 10a through the nucleus-to-dorsal growth axis of the otolith shows a plate-like crystal organization in two planes within the otolith. A similar broken section from another otolith shows the three-dimensional aspect of the plate-like crystal growth that is formed by a block-like crystal growth unit (Fig. 10b).

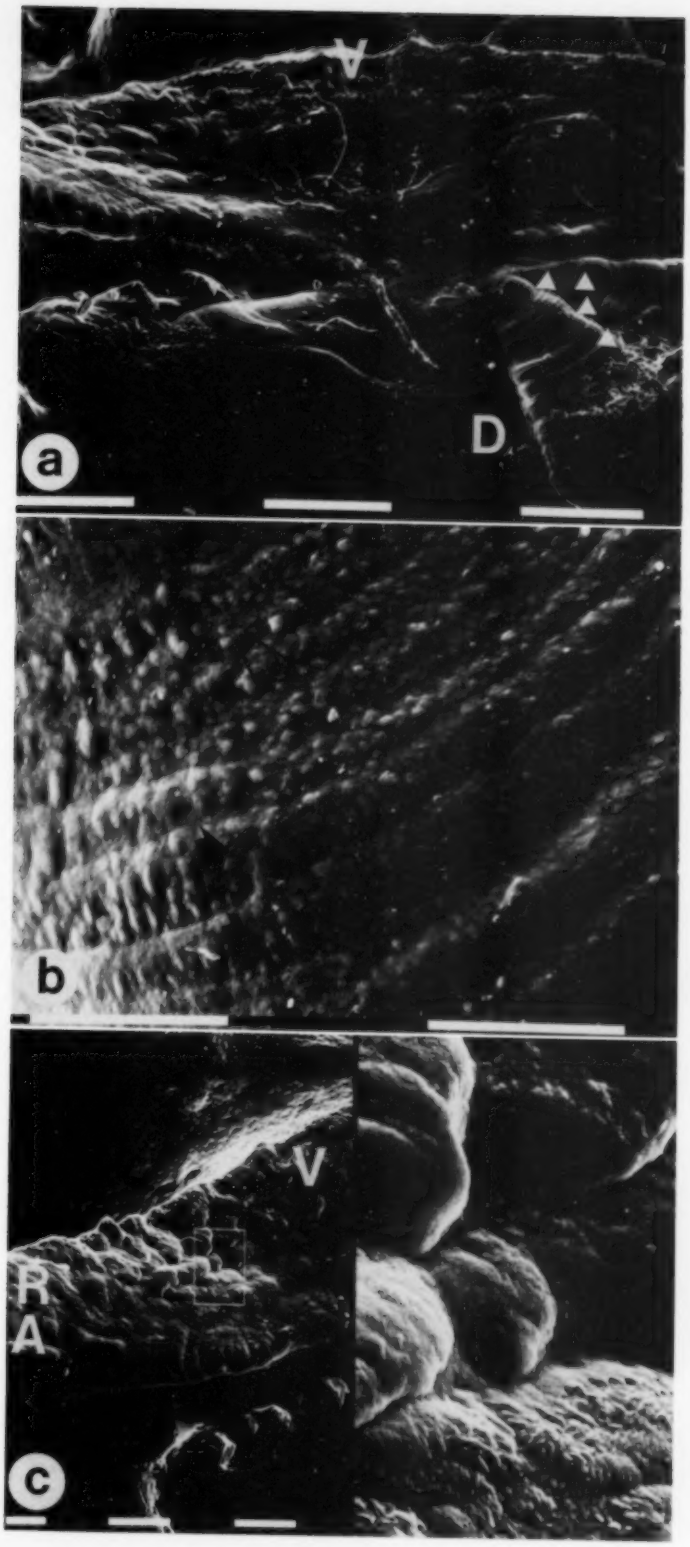
Polished and Etched Sections of Otoliths

Polished sections of otoliths usually reveal more of the features that are of interest in age estimation. The otolith of *Nemadactylus macropterus* is fairly flat in the dorso-ventral plane and can be ground and polished in both the horizontal and vertical sections.

Horizontal Sections

The otolith of *Nemadactylus macropterus* has a complex check structure in the horizontal plane. The most striking feature is a long segment that runs from the nucleus to the ventral anterior end of the otolith (Fig. 11a). A shorter, less well delineated segment runs to the posterior dorsal end of

FIG. 6. (a) SEM of the antisulcal surface of the otolith of *Nemadactylus macropterus* showing a qualitative difference in surface sculpturing between the ventral (V) and dorsal (D) sides of the otolith; sculpturing along the posterior-dorsal prisms shows a wide range of widths (arrows). Scale bar = 1 mm. (b) The most narrow intervals of the surface sculpturing (steps about 3.5 μm wide, arrows), composed of granular crystals (open arrow); bar is 10 μm. (c) ventral anterior edge (V, A) shows a series of coarse zones that become smoother as they pass into the anterior ramus (R). The right-hand side shows an about 7× magnification of the box in the left hand side showing the block-like crystals that form the coarse zones of the ventral edge; scale bar = 0.5 mm.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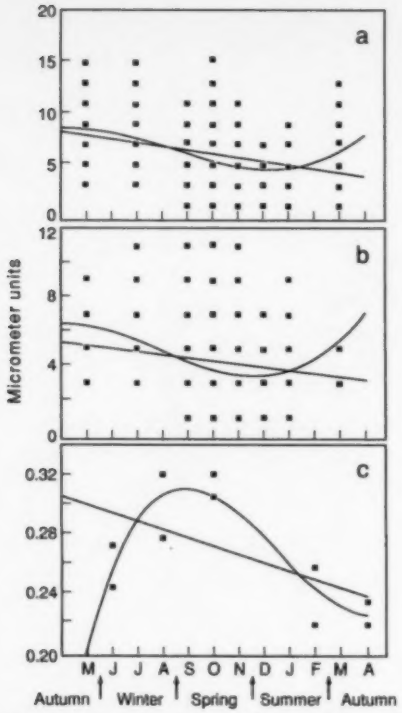


FIG. 7. Marginal increment widths of the otolith of *Nemadactylus macropterus* show a linear decrease over the austral spring and summer. The polynomial curve shows an apparent annual periodicity with a minimum in the austral spring and summer. Fitted curves are shown on the graph. A number of different observations of marginal increments were made resulting in the scatter of points. Units are ocular "micrometer" units. (a) Age group 4 of Tong and Vooren (1972). (b) Age group 5 of Tong and Vooren (1972). (c) Data compiled from Smith (1982).

the otolith. The ratio of lengths of the posterior/anterior segments is 0.53, which is close to the macula posterior/anterior segment ratio.

A large number of checks appear between the nucleus and the ventral edge of the otolith. Although it may be possible to divide these checks into major and minor checks, it is evident that near the edge of the otolith such a distinction would break down. Counting all checks, there are about 30 checks between the nucleus and the ventral edge. Similarly, counting along the recurved axis from the nucleus to dorsal prisms shows 30 checks, if any division into major and minor checks is ignored (Fig. 11b). An unknown amount of material is lost at the edge in grinding the otolith and so cumulative widths of checks by distance indicate the progressive growth of the otolith rather than its true size. The pattern of growth is different along the dorsal and ventral axes. The cumulative growth curves from internal checks are different from the growth curves that are based on cumulative widths of opaque zones observed on the antisulcal surfaces of the whole otoliths shown in Figs. 5a and 5b, even though counts were made along the same axis.

Radiating microincrements of the type described as daily in Campana and Neilson (1985) surround the single nucleus in Fig. 11a and can be seen at high magnification in Fig. 12a. Microincrements are visible in both the nucleus dorsal prism and nucleus–ventral axes (Fig. 12a). The first 8 microincrements are narrow (average width 0.98 μm), the next 32 are wider (average width 2.72 μm) and those that follow, narrower (average width 1.75 μm). The etched section in Fig. 12a shows areas that have no apparent microincrement sequences as well as narrow bands of etch-resistant material. Microincrement widths are fairly constant along the nucleus – dorsal anterior growth axis, averaging about 1.8 μm in width (Fig. 12b). The microincrements of the otoliths of *Nemadactylus macropterus* are poorly differentiated in terms of etch resistance. The SEM in Fig. 12b has been severely etched, with consequent damage to the underlying crystal structure, but with the microincrement structure remaining weakly differentiated.

Microincrement sequences in the otolith of *Nemadactylus macropterus* vary in their interactions with both apparent and real discontinuities giving rise to both apparent and real checks. For example, in Fig. 11a, low-magnification views indicate a check at the point A on the nucleus – ventral growth axis. However, in Fig. 12a there is no indication of a discontinuity in the microincrement sequence that ought to occur with a check in growth. This particular check is an example of a magnification-dependent check.

In other parts of the otolith of *Nemadactylus macropterus* the relationship between microincrements and checks is more complex. For example, in Fig. 12c one check has a continuous microincrement sequence growing past it, even though the same microincrements apparently grow into the check itself. The overall effect is that measurements of direct linear distance that crosses checks will result in an underestimate in the number of microincrements laid down if the linear distance is divided by the average microincrement width in order to estimate microincrement number. Similar "losses" in the estimation of age by average microincrement count occur in the otolith of *Macruronus novaezelandiae* (Gauldie 1993). The difference in microincrement counts in the two pathways shown in Fig. 12c amounts to a loss of about 6 microincrements for every 0.1 mm of measured distance. Repeating the same procedure on five further checks generated an average loss of about 5 microincrements for every 0.1 mm of measured distance along the nucleus – dorsal prism growth axis. Age estimates based on integrated microincrement width over the length of the otolith growth axis require correction for the loss of microincrements due to check effects.

Vertical Sections

A vertical section of the dorso-ventral axis cut through the nucleus of an otolith of *Nemadactylus macropterus* is shown in Fig. 13a. Examined by transmitted light, there is a darker region around the nucleus followed by a light zone and then a less dark zone with a final light zone going to both dorsal and ventral edges. The same section viewed by reflected light (Fig. 13b) shows the reversal of light and dark zones that one might expect from the difference in refractive index implied by the transmitted light image. Light zones viewed by reflected light are much wider in section than in whole otoliths. In addition, the ventral side of the otolith shows a tendency for multiple zones that is absent on the dorsal side. The material that fills in the sulcus appears lighter by reflected light (Fig. 13b). When the same section is lightly etched and observed by transmitted light (Fig. 13c) it is evident that the tendency for multiple

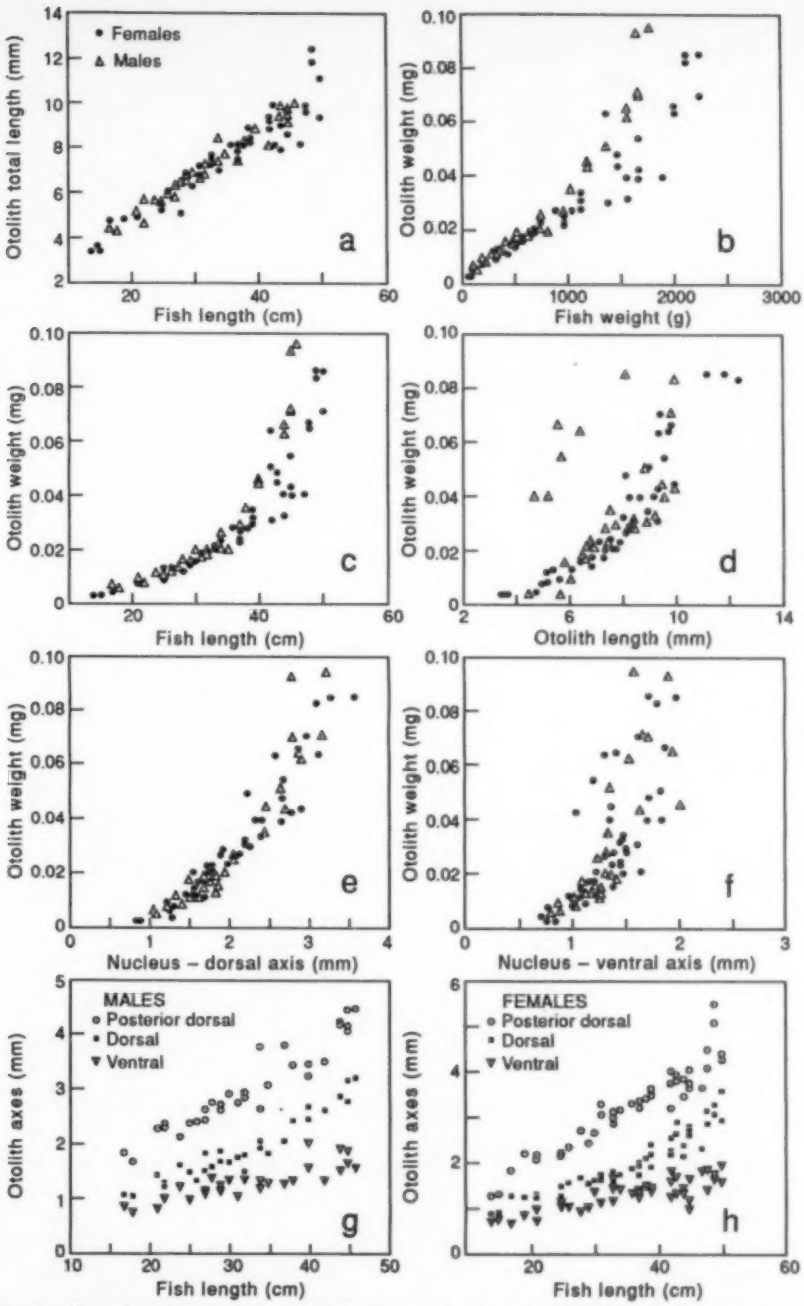
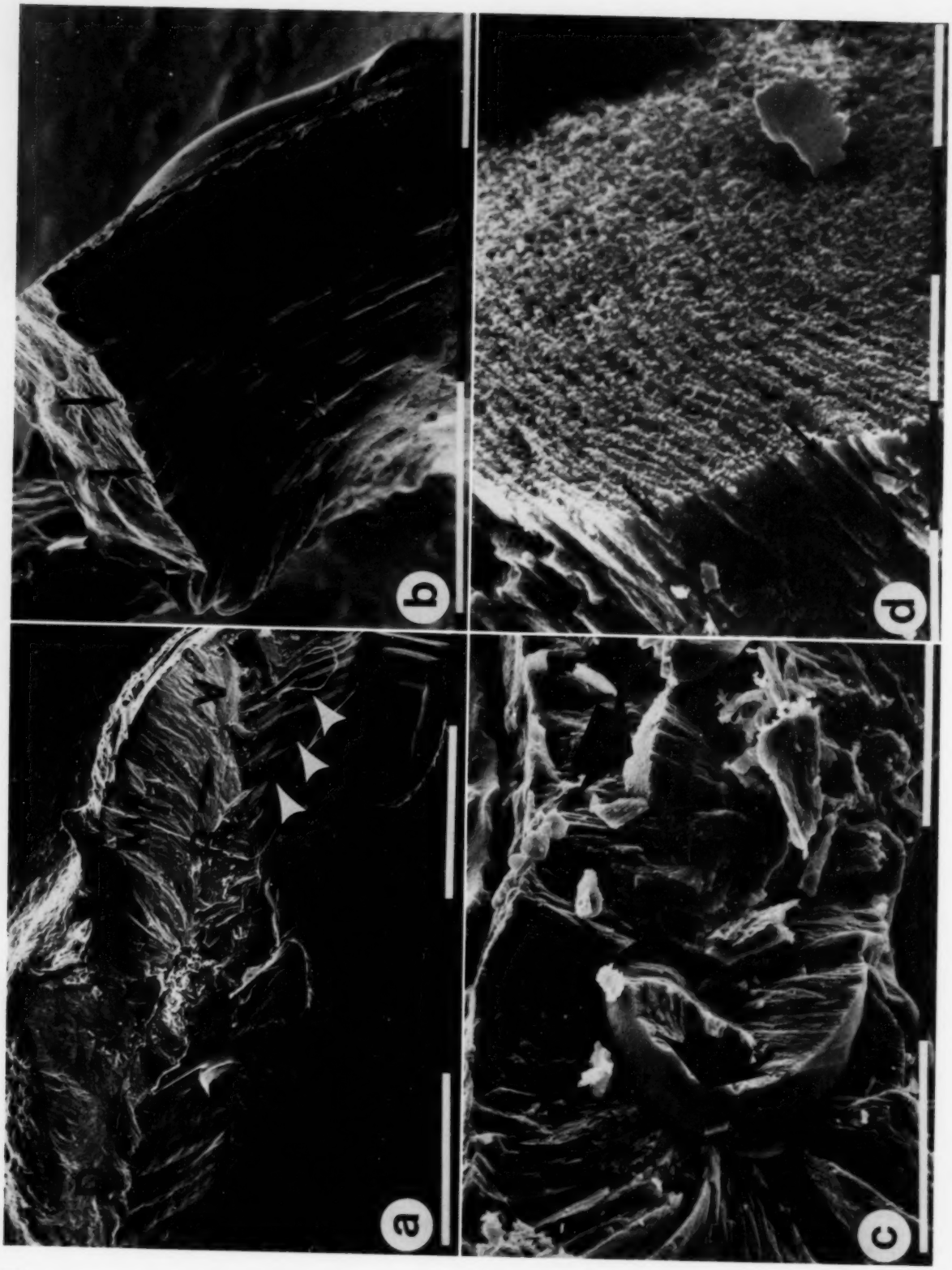


FIG. 8. Otolith variables for *Nemadactylus macropterus* plotted against whole-fish or other otolith measurements. (a) Otolith length against fish length: (b) Otolith weight against fish weight. (c) Otolith weight against fish length. (d) Otolith weight against otolith length. (e) Otolith weight against the nucleus–dorsal axis length. (f) Otolith weight against the nucleus–ventral axis length. (g) Otolith axis lengths against fish length for males. (h) Otolith axis lengths against fish length for females.

zones observed by reflected light reappears in the difference in etch sensitivity between the dorsal and ventral sides of the otolith.

The three views of the same section in Fig. 13 reveal many of the problems of interpretation of patterns of zones and checks in the otolith of *Nemadactylus macropterus*. All three sections are marked with arrows at the antisulcal surface at the points that would correspond to opaque zones on the antisulcal surfaces when the otoliths are observed by reflected light. It is evident that such opaque zones would be entirely out of phase with opaque zones observed in sections. In addition, opaque and hyaline zones in sections are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of similar width, whereas the hyaline zones observed in whole otoliths by reflected light are only a small fraction of the width of opaque zones. The same problems apply to checks in both the antisulcal and sulcal growth axes of the otolith. There is an order of magnitude difference in the number of checks that could be assigned to the same otolith section depending on the method of preparation.

Microincrement Distribution Along the Growth Axes of the Otolith

Microincrements of the kind widely described in the literature (e.g., Campana and Neilson 1985) as daily microincrements occur in both horizontal and vertical sections of the otolith of *Nemadactylus macropterus* both in etched and optical sections. Microincrements present a number of problems in the otoliths of *N. macropterus* which include their distribution along the different growth axes of the otolith, and validation of daily microincrementation.

Microincrement width distribution along the growth axes of the otoliths can be used to age fishes directly (Jones 1986) or from the relationship between microincrement width and the length of the growth axis of the otolith (e.g., Smith and Kostlan 1991; Ralston and Williams 1988). Microincrement widths were measured at the edges of the dorsal and ventral growth of 107 otoliths of *Nemadactylus macropterus* by taking the average of 10 microincrement widths as close as possible to the edge of the otolith. Recovering microincrement widths from optical, rather than ground, sections of the edge of whole otoliths is not the usual procedure, which usually involved polishing and/or etching. The range of availability of microincrement images recovered using this method is shown in Fig. 14. Repeated samples (n = 10) were made of successive groups of 10 microincrements in two otoliths whose image quality ranged from best to worst. The average coefficient of variation of mean increment width of the ten repeated samples was 19.2% and ranged from 17.9 to 20.5%.

Measurements of both the dorsal and ventral growth axes were made (using an ocular micrometer) as the linear distance from nucleus to the edge of the posterior dorsal prism, and to the ventral edge of the otolith. The larval and juvenile life history of *N. macropterus* is complicated by a long larval phase (Vooren 1972) and otoliths of juvenile and larval fishes below 14 cm FL were unavailable for this study. Instead, measurements of microincrement widths were made from ground sections in the first 1 mm of axial growth in the otolith. Microincrement width was plotted against the lengths of the nucleus – posterior dorsal axis (Fig. 15a), and the nucleus – ventral axis (Fig. 15b).

Straight lines were fitted through all of the data in Figs. 15a and 15b to represent a relationship between microincrement width and axial length. There is no theoretical relationship published between microincrement width and axial length, nor are the causes of variation of microincrement well understood, so a statistical best-fit straight line is a simplification. Nonetheless, the studentized residuals are normally distributed around the fitted curve suggesting that a straight line adequately describes the underlying relationship implied in these data. The coefficient of variation of the spread in data around the fitted line is about 20%. The variation in microincrement widths between otoliths is therefore of the same order of magnitude as the variation in microincrement widths within continguous parts of otoliths. In both Figs. 15a and 15b the r^2 values indicate that only about 10% of the variation in microincrement width is explained by change in axial length. Axial lengths are weakly related to each other (r^2 = 0.25; Fig. 15c) and microincrement widths in both the nucleus–ventral and nucleus – posterior dorsal axes are weakly related to each other (r^2 = 0.2; Fig. 15d).

There is considerable scatter in the microincrement width – axial distance plot. Along the nucleus – posterior dorsal axis the ratio of standard error to fitted slope parameter for microincrement width was 27%. The residuals were normally distributed around the fitted line, but the high coefficient of variation shows that caution is required in using small (i.e., less than 30) samples of otoliths to estimate length-at-age curves.

The wide scatter observed in microincrement width occurs in an environment that is probably always saturated with respect to calcium because of the continuous availability of otoconia in the endolymph. Under these circumstances, it appears unlikely that variation in microincrement width is due to changes in the short-term supply of calcium to the endolymphatic sac.

Microincrement widths along the ventral growth axis showed a different distribution than those along the dorsal growth axis. In smaller otoliths, ventral microincrements had about the same average width as dorsal microincrements, but with increasing size the ventral microincrements became, on average, wider, and with greater variability in width. In the largest otoliths, ventral microincrements could not be distinguished from the large number of checks near the ventral edge checks and were not measured.

In an attempt to validate daily microincrementation both wild and tank-held specimens of *Nemadactylus macropterus* were injected with oxytetracycline and tagged. Mortalities were high. None of the specimens injected in the wild with oxytetracycline and tagged were recovered. Specimens held in aquaria had high mortalities, averaging 22 days of survival in captivity after being caught by trawl, tagged, and oxytetracycline injected. Otoliths from specimens held in aquaria were examined by UV light and showed oxytetracylcine uptake on the surface of the sulcal side of the otolith, none on the surface of the anti-sulcal side, and no growth following tetracycline uptake, or so little growth as to not be discernable by light microscopy. Testing microincrementation periodicities in the otolith of *N. macropterus* must therefore be done indirectly from age estimates based on microincrement width data.

FIG. 9. Broken sections of an otolith of *Nemadactylus macropterus* (a) broken through the nucleus along the dorso–ventral axis (D, V). Sculpturing on the antisulcal surface (white arrows) does not show any structural correlates within the otolith. The internal structure shows radial (R arrow) and medial (M arrow) crystal growth. Scale bar = 1 mm; (b) Detail of the sculptured antisulcal surface shows no relation between surface features (arrows) and the internal structure. Scale bar = 1 mm; (c) Spalling surfaces (arrows). Scale bar = 0.1 mm; (d) Certain spalling surfaces are associated with a plate-like (arrows) underlying crystal organization. Scale bar = 10 μm.

FIG. 10. Broken sections of otoliths of *Nemadactylus macropterus* (a) showing a complex two-dimensional plate-like structure with periodic structures (arrows) in two planes that are 1–2 μm wide. Scale bar = 10 μm. (b) Detail of the plate-like crystals showing that the underlying periodic structure is organized in two planes and is composed of small block-like crystals (arrows). Scale bar = 10 μm.

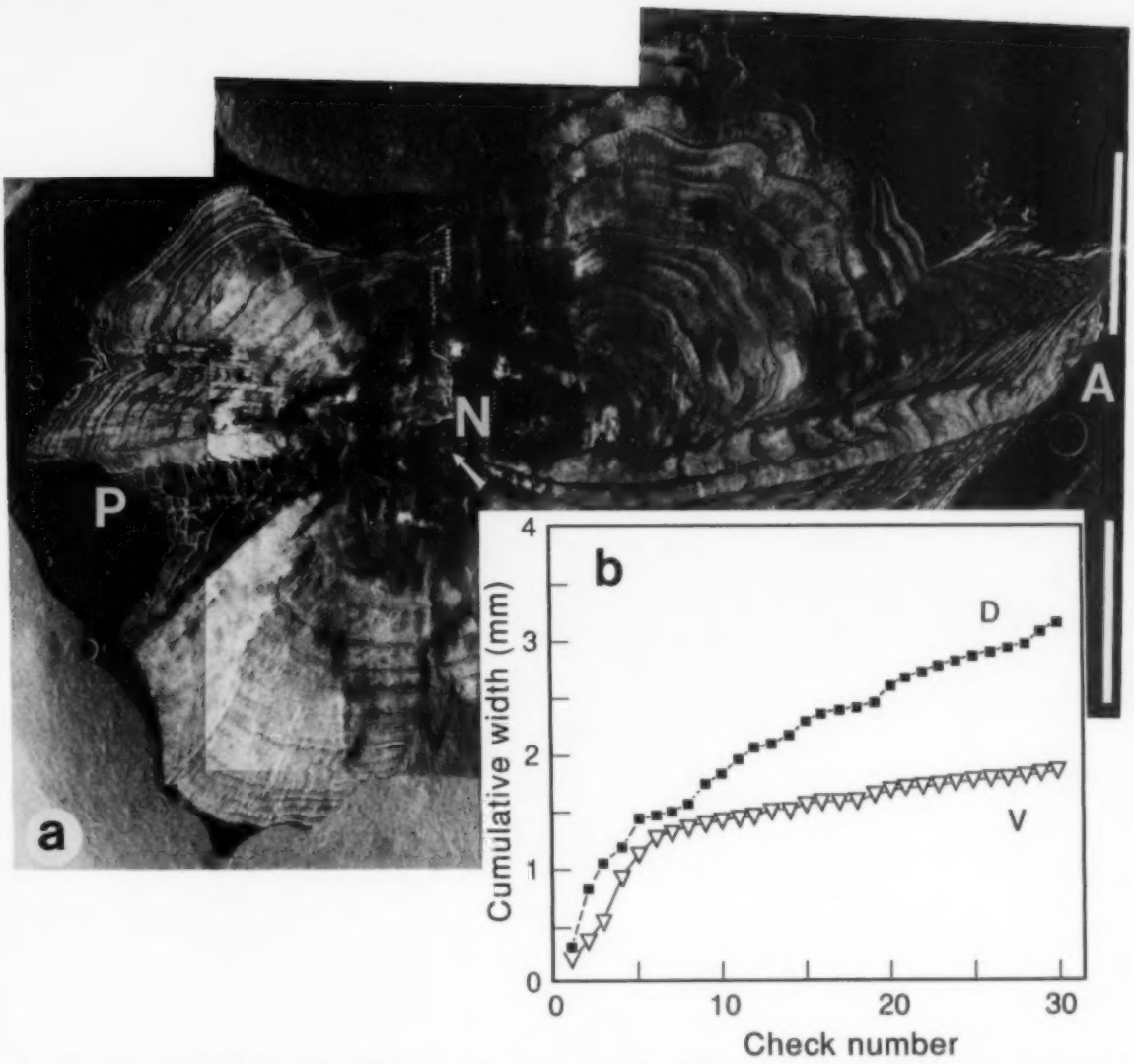
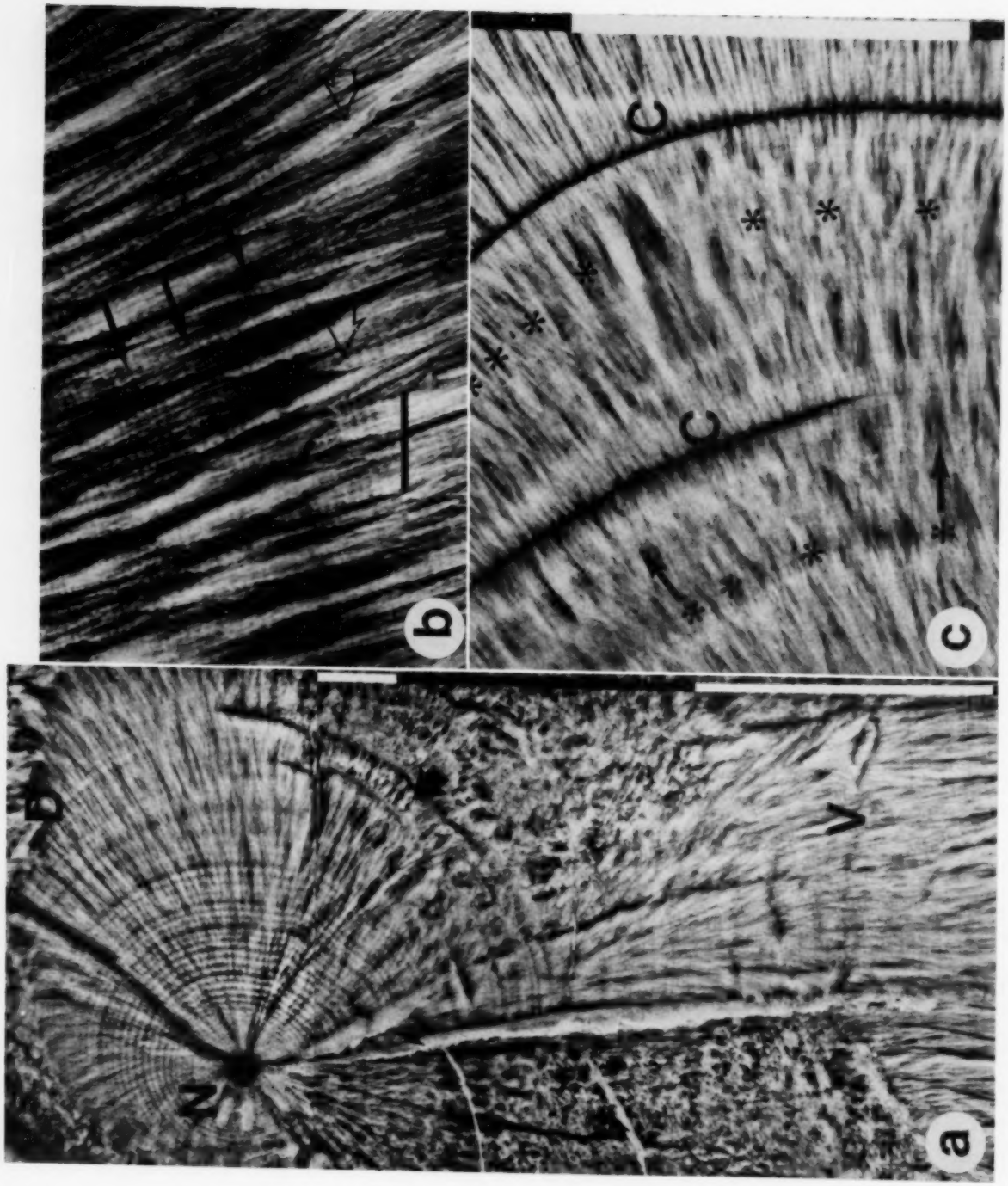


FIG. 11. (a) A composite SEM of a polished horizontal section of the otolith of *Nemadactylus macropterus*. Segments run from the nucleus (N) to the anterior ventral (A and V) and posterior dorsal (P and D) parts of the otolith (the latter is less clearly delineated). A pseudo-check near the nucleus is shown with a white arrow. (b) Cumulative widths of dorsal (D) and ventral (V) checks in the horizontal section of the otolith of *N. macropterus* plotted by check number from the nucleus at zero.

Estimation of Age From Microincrements of *Nemadactylus macropterus*

Microincrement-based ages were derived following the general method of dividing the length of the otolith growth axis by the average microincrement width obtained by integrating from the general relationship between microincrement width and axial length. The nucleus – posterior dorsal growth axis is usually recurved to the anterior as it grows from the nucleus to the dorsal edge (e.g., Fig. 4a). Linear measurements of the nucleus – posterior dorsal length axis required corrections for this curvature. The curvature was slight and resulted in an average 2.7% increase in the corrected length. Similarly, the loss of microincrements due to checks required a correction. On average, a loss of 5 increments per 0.1 mm resulted in a 9% underestimate of true age. Both corrections were applied to age estimates based on microincrement width. Length at age for *Nemadactylus macropterus* using the microincrement technique shown in Fig. 16 was based on the corrected nucleus – posterior dorsal growth axis. The distribution of lengths-at-age in Fig. 16 has been fitted with a lowess curve (Cleveland 1979). There were no significant differences between males and females observed in this data set.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Testing Microincrement Periodicities

Adult *Nemadactylus macropterus* do not show modal length frequencies. Tong and Vooren (1972; p. 19) state that "Length–frequency distributions of catches of adult tarakihi taken on the commercial fishing grounds have never been found to show modes indicating individual age groups."

However, juvenile *Nemadactylus macropterus* show distinct length modes in catches made on their nursery grounds (Tong and Vooren 1972; Vooren 1972; Vooren 1975). Juveniles show two, sometimes three, length modes corresponding to the first three year-classes. It is these two or three length modes that provide evidence to support the interpretation of otolith zone ages in Tong and Vooren (1972). Comparison of the microincrement length-at-age curve in Fig. 16 with modal lengths of juvenile groups in Tong and Vooren (1972; Fig. 7, p. 21) and Vooren (1975; Fig. 1b p. 147) shows substantial agreement in length-at-age over the first three years. Listing modal lengths from Tong and Vooren (1972), Vooren (1975), and the lengths estimated from microincrement ages, the agreements are as follows (in cm):

	Year 1	Year 2	Year 3
Microincrement	9	17	23
Tong and Vooren (1972)	9	15	22–25
Vooren (1975)	10	17	22

In a paper dealing with juvenile *Nemadactylus macropterus*, Vooren (1972) described and measured otoliths of postlarvae and juveniles from the length modes corresponding to first and second years. Vooren (1972) reported otolith "diameter" (Vooren 1972; pp. 611, 612) from which the dorsal growth axis can be estimated as "radius." Vooren (1972) reported 25 specimens of 63 to 80 mm fork length as having otolith radii ranging from 0.39 to 0.515 mm, with a mean of 0.44 mm from specimens of *N. macropterus* caught in the southern part of the South Island of New Zealand. The microincrement curve in Fig. 15a indicates that these radii would correspond to a range of ages from 229 to 303 d with a mean of 259 d. These fish were caught on 4 December 1968, so "microincrement" ages correspond to dates of birth centered in late February ranging from January to March 1968. The spawning grounds nearest to the point of sampling by Vooren (1972) were reported by Tong and Vooren (1972) as having spawning dates: "January–February onward?"

Vooren (1972) reported a further group of 50 specimens of 110–149 mm fork length with otolith radii ranging from 0.68 to 0.87 mm, with a mean of 0.795 mm, that were caught in Tasman Bay on 8 July 1969. These otolith radii correspond to ages ranging from 400 to 511 d with a mean of 468 d. These ages would correspond to spawning dates ranging from March to May 1968, centered on mid-April. The nearest spawning ground to Tasman Bay reported in Tong and Vooren (1972) is at East Cape, for which they report the spawning period as April. The close correspondence between spawning dates reported by Vooren (1972) and Tong and Vooren (1972) and dates back-calculated from otolith "radii" using average microincrement widths provides support for the assumption that microincrement deposition in the otolith during the early life of *Nemadactylus macropterus* is daily.

Discussion

The anatomy of the inner ear of *Nemadactylus macropterus* is of the general teleost type (e.g., Dale 1976) in which the otoliths are attached by a membrane to the macula. Although the relative dimensions of the otolith of *N. macropterus* change with increasing size, the relative dimensions of the anterior and posterior parts of the macula that support the otolith do not change with increasing size. There is not, therefore, any evident relationship between the shape of the macula and the shape of the otolith. The exposure of the kinocilia to the endolymph requires that calcium ion concentration of the endolymph is buffered. The endolymphatic sac of *N. macropterus* contains enough calcium in the form of otoconia to buffer the endolymph against reduction in calcium ions for at least a few days. Consequently, one might expect microincrement deposition to also be buffered against changes in calcium ion concentration in the endolymph.

In a morphological sense, all of the periodic structures in the otolith are related to each other. However, the periodicities off these structures differ, so it is difficult to reconcile apparent ages derived from different structures. The basic problems of estimating ages of *Nemadactylus macropterus* from their otoliths are demonstrated in the different length-at-age curves resulting from different methods. Zone methods of ageing yield maximum ages of 20+ (Tong and Vooren 1972), and 16+ (Smith 1982), with ages up to 42+ for sulcal checks (Vooren 1977), compared with maximum ages of 9+ from the daily microincrement method. Maximum ages of *N. macropterus* based on scales (Han 1964) were similar to microincrement-based ages: 9+ for females at 40.3 cm FL and 7+ for males at 37.8 cm FL. Scale and otolith ages are often different (O'Gorman et al. 1987) making comparisons of otolith and scales ages in *N. macropterus* difficult without more information than the brief note of Han (1964) provides. However, the large discrepancies in otolith-based age estimation methods are too great to be reconciled as sampling or approximation effects. It is necessary to examine the arguments used in justifying each method.

The Zone Method of Tong and Vooren (1972) and Smith (1982)

Two methods were used by Tong and Vooren (1972) and Smith (1982) to argue that the opaque and hyaline zones of whole otoliths were annual events: the method of marginal increments and correspondence with the first 2–3 length modes in catch curves.

FIG. 12. An etched horizontal section of the otolith of *Nemadactylus macropterus* (a) showing a well-defined single nucleus (N) surrounded by radiating microincrement sequences growing in both the nucleus–dorsal (N, D) and nucleus–ventral (N, V) axes. Etch resistant bands are indicated by small arrows. The location of the pseudo-check is shown by a large arrow. Scale bar = 100 μm; (b) Showing regular microincrement sequences (arrows) even though etching severely disrupts the crystal structure (open arrows) of the otolith. Scale bar = 20 μm. (c) Showing two well-defined checks (C). Microincrements can be seen growing into the checks (arrows). Two microincrement lines on either side of the upper check have been marked (*) to allow counting along the continuous microincrement sequence on the left-hand side and counting across the microincrement sequence interrupted by the upper check. Scale bar = 20 μm.

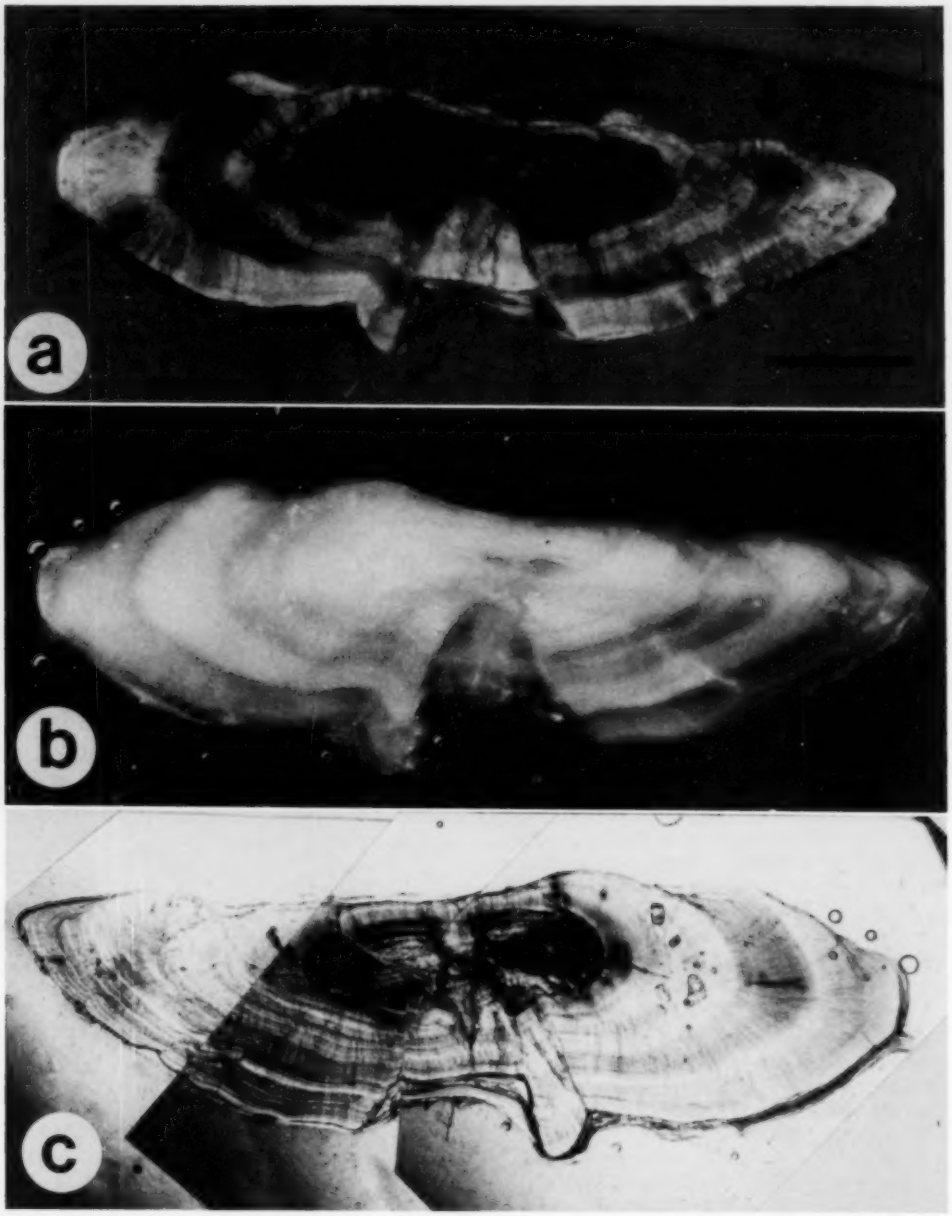

FIG. 13. The same dorso-ventral section of the otolith of *Nemadactylus macropterus* shown in three different treatments: (a) transmitted light; (b) reflected light; and (c) etched and viewed by transmitted light. Arrows (a) indicate the sculpturing on the antisulcal surface that results in opaque zones in whole otoliths. Scale bar (a) = 0.5 mm.

The method of marginal increments depends on the recognition of annual opaque and hyaline zones in whole otoliths of *Nemadactylus macropterus*. Opaque and hyaline zones of whole otoliths of *N. macropterus* result from surface sculpturing. Internal opaque and hyaline zones also occur, but they are out of phase with surface sculpturing and have a different pattern of deposition. The basic assumption that sustains the use of opaque and hyaline zones is that a broad opaque zone is laid down during the summer and a narrow hyaline zone is laid down during the winter period of low growth. Internal opaque and hyaline zones show similar widths, not a broader opaque zone and a more narrow hyaline zone. There is no evidence to support the assumption that opaque and hyaline zones in the otolith of *N. macropterus* are annual events. Furthermore, the opaque and hyaline zone patterns in whole otoliths of *N. macropterus* are contradicted by opaque and hyaline zone patterns in sections of otoliths. The same contradiction has been described in otoliths of other species (Gauldie 1987). In addition, the resolution of the opaque and hyaline zones of whole otoliths

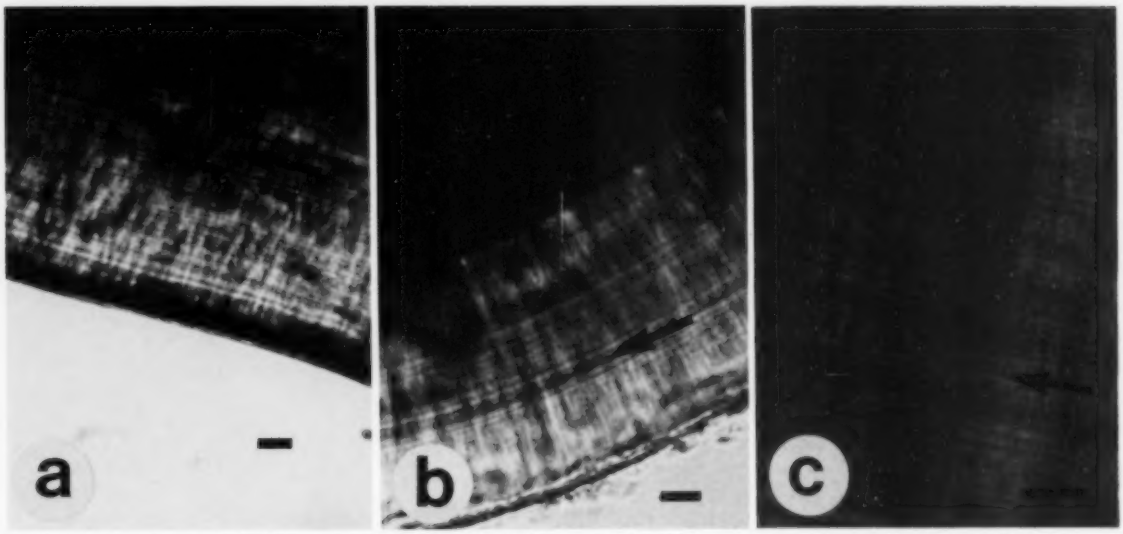

FIG. 14. Optical sections of microincrements shown for three different otoliths of *Nemadactylus macropterus* that range in image quality. Microincrements are indicated by arrows. Scale bars = 10 μm.

is magnification-dependent. The problems of both magnification dependence and subjectivity of opaque and hyaline zone recognition was acknowledged by Tong and Vooren (1972 p. 6; para. 3): "To distinguish checks from true zones the overall pattern of the otolith has to be looked at under low magnification." Smith (1982) acknowledges similar difficulties, particularly in older otoliths. It is unfortunate, but a hyaline increment will remain hyaline even when increasing magnification decreases the width of the apparent marginal increment. Thus the same observation of a hyaline zone could be used to "validate" many different ages, depending on magnification.

It is evident that the use of marginal increments to validate ages by showing that the broad opaque zone does in fact grow in the summer is already compromised by the problems of recognizing a true opaque zone and therefore its time of formation. Smith (1982) notes that his marginal increment "plots shown are mostly a combination of both hyaline and opaque edges and it is therefore difficult to determine a specific time for ring formation." Plotting Smith's (1982) mean marginal increment data from consecutive austral autumn to austral autumn showed that the "opaque zone" decreased in width over summer. Exactly the opposite of what is required for validation of age assignment by the pattern of opaque and hyaline zones.

Tong and Vooren (1972; Fig. 11, p. 25) presented "monthly mean width of 'recent layer' on the otoliths" plotted by month of capture to support the claim for growth of the broad opaque zone in the austral summer. However, replotting the original marginal increment data supplied by Tong and Vooren (1972; Fig. 5, p. 18) showed the same effect as observed in the marginal increment data of Smith (1982), a decrease in opaque zone width over the austral spring and summer.

It is not uncommon in fisheries research for contradictory interpretations to be made from the same data. Hypothesis testing is, after all, often only a procedure of offering different interpretations. Studies of marginal increment width in whole otoliths of other species show an appropriate increase in opaque material over the summer, but often the opaque material does not develop in all fish. For example, Pinhorn (1969) reported data for *Gadus morhua* in which about 60% of immature fish and about 30% of mature fish did not appear to develop an opaque zone at all. Re-investigation of the problem using marginal increments based on internal opaque and hyaline zones may be more productive, as there is direct evidence in some species to support seasonal periodicities of such zones (e.g., Radtke et al. 1985). However, the pattern of growth of the otolith resulting from cumulative check ring width varies in different axes of the same otolith. In addition, the dependence of check rings on magnification can radically change the number of "steps" in the cumulative check ring width curves, depending on the choice of magnification. Fish size (weight and length) is related to otolith size (weight and length for different axes) so that different cumulative check width curves lead to different ages and differently shaped growth curves.

The length distributions of *Nemadactylus macropterus* show modalities in the length distributions of juveniles but not those of adults. The changes in length distributions of juveniles over time support their modalities as indicators of fish age. Using juvenile length modalities, Tong and Vooren (1972) confirm their age assignment for the first 2–4 years of *N. macropterus* as being based on acceptable assumptions. Without the support of the marginal increment methods, the extrapolation of ages of *N. macropterus* from 2–4 to 20+ by Tong and Vooren (1972) is based on consensus from the general ageing literature and not from direct evidence.

The Check Method of Vooren (1977)

Ages of *Nemadactylus macropterus* were extended from 20+ to 45+ by Vooren (1977; p. 4, para. 2) by changing the basis of the age estimation procedure from opaque and hyaline zones of whole otoliths, to sulcal checks in cross sections of otoliths with the following justification: "After tarakihi reach an age of about 15 yr, further growth zones are not laid down uniformly around the otolith, but mostly on its convex side; from then on the otolith becomes progressively

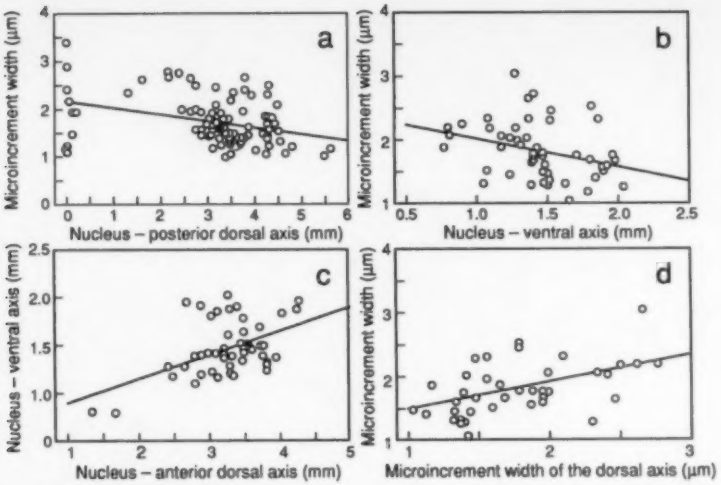

FIG. 15. *Nemadactylus macropterus* otolith measurements: (a) Microincrement widths (μm) plotted against the length of the nucleus – posterior dorsal axis (mm). (b) Microincrement width (μm) plotted against the length of the nucleus–ventral axis (mm); (c) Nucleus–ventral axis length (mm) plotted against nucleus–posterior dorsal axis length (mm). (d) Microincrement width (μm) in the nucleus–ventral axis plotted against microincrement width (μm) of the nucleus–posterior dorsal axis.

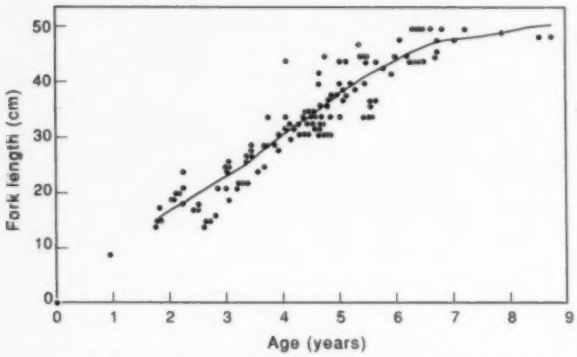

FIG. 16. Fork length (cm) plotted against age in years estimated from microincrement width. The fitted line is a lowess smoothing curve.

thicker with age but its width and length increase little. The annual growth zones are thus difficult to distinguish on the whole otolith, but they can be seen clearly by examining a burnt cross-section of the otolith, using the method devised by Möller Christensen (1964)."

Vooren (1977) did not offer data to support the check method of age estimation. The relative increase in otolith weight observed in this study is consistent with the change in weight due to increase in volume. The growth axes of the otoliths of *Nemadactylus macropterus* continue to grow with increasing size of the otolith. The ventral axis of the otolith grows more slowly than the other axes, but all of the axes of the otolith continue to grow over the life of the fish.

Sulcal check patterns are complex in the otolith of *Nemadactylus macropterus*, and without some explanation of check patterns age assignment is not possible. Other parts of the otolith also have complex check patterns. Horizontal sections of the otoliths have many checks, and the curves of cumulative widths between checks plotted against axial length are similar in shape to growth curves described for many species of fish, including those of *N. macropterus*. Some checks are only partial discontinuities in the continuous growth of the otolith, but some checks may represent temporary cessation of growth. However, check (and zone) patterns vary from axis to axis, from with the result that different parts of the otolith may yield different ages.

The method of Vooren (1977) was based on the consensus of opinion in the general ageing literature on appropriate techniques for ageing fish rather than direct supporting evidence.

The Daily Microincrement Method

Ages assigned to otoliths of *Nemadactylus macropterus* from microincrements in the nucleus – posterior dorsal otolith growth axis give lengths-at-age that match those anticipated from length modalities that are thought to represent the first 1–3 year-classes. In addition, otolith radii of juveniles published in the literature allowed back-calculation of spawning dates from average microincrement widths that matched known spawning dates. Age assignments from daily microincrements for the first 1–3 year-classes are supported by observation, but extrapolation of daily microincrement ages from 1–3 years to 9+ years is not supported by any direct evidence.

In addition to microincrement deposition in the nucleus – posterior dorsal growth axis, microincrements also occur in the nucleus – ventral growth axis of the otolith of *Nemadactylus macropterus*. The microincrements of the nucleus – ventral growth axis differ in size, size distribution, and rate of change with axial length from those in the nucleus – posterior dorsal axis and would lead to different ages if they were assumed to be daily. At the micrometre level of resolution, the otolith of *N. macropterus* represents a very large space. It is possible that an exhaustive search of the otolith would reveal many microincrement-bearing growth axes, each with the potential for a different age. However, the

nucleus – posterior dorsal axis consistently shows the most growth relative to the other axes of the otolith. Therefore the nucleus – posterior dorsal axis can be regarded as having the potential for the longest continuous sequence of microincrements and therefore provides the most conservative estimate of age.

Microincrements in both the nucleus – posterior dorsal and nucleus – ventral growth axes are only weakly correlated to axial length. This is consistent with the clock-like mechanism implied by the assumption of daily microincrementation. The otoconia of the macula of the otolith of *Nemadactylus macropterus* provide a sufficient reservoir of calcium carbonate to effectively buffer the growth of the otolith against short-term gradients in plasma calcium. This kind of buffering potential would make microincrementation less dependent on axial length as well as making it difficult to argue for an organismic cause for cessation of microincrementation. Studies in other species have shown that microincrementation in the otolith is very resistant to physiological stress (Mugiya and Uchimura 1989), although microincrement width (and even microincrement opacity) may be related to somatic growth rates (Radtke et al. 1985).

Conclusions

Age determination of *Nemadactylus macropterus* is a significant component of the fisheries management strategy for this species (Vooren 1973; Vooren 1977; Smith 1983; Wankowski 1988). Given the range of different ages offered, it is useful to summarize the arguments used to support each method.

	Zones	Check	Microincrements
Determination of pattern	subjective	subjective	quantitative
Errors	subjective	subjective	quantifiable
Back-calculation of birthdays	none	none	positive
Correlation with length modes	positive	positive	positive
Extrapolation from verifiable ages	7:1	14:1	3:1

Otoliths are complex structures for which the relations between otolith morphology (which includes zones, checks, and microincrements) and otolith function are poorly understood. Nonetheless, presented in the form of the table above, the support for the microincrement age estimation procedure of otoliths of *Nemadactylus macropterus* is stronger than that for either the zone or check method. Application of the microincrement aging method in specific samples of *N. macropterus* requires (*i*) confirmation by spot-tests of the underlying microincrement width distribution in the otolith, and (*ii*) a sufficient sample size to ameliorate the uncertainty in age estimation due to the inherent scatter in microincrement width.

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References

AYLING, T., AND G.J. COX. 1982. Collins guide to the sea fishes of New Zealand. Collins, Auckland and London.

BEAMISH, R.J. 1979. Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. J. Fish. Res. Board. Can. 36: 141–151.

BEAMISH, R.J., AND G.A. MCFARLANE. 1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112: 735–743.

CAMPANA, S.E., AND J.D. NEILSON. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42: 1014–1032.

CLEVELAND, W.S. 1979. Robust locally weighted regression and smoothing scatter plots. J. Am. Stat. Assoc. 74: 829–836.

DALE, T. 1976. The labyrinthine mechanoreceptor organs of the cod *Gadus morhua* L. (Teleostei: Gadidae). Norw. J. Zool. 24: 85–125.

DANNEVIG, A. 1933. On the age and growth of the cod (*Gadus callerias* L.) from the Norwegian Skagerrack coast. Fiskeridir. Skr. Ser. Havunders. 4.

DEGENS, E.T., W.G. DEUSER, AND R.L. HAEDRICH. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. 2: 105–113.

DUNKELBERGER, D.G., J.M. DEAN AND N. WATABE. 1980. The ultrastructure of the otolith membrane and otolith in the juvenile mummichog, *Fundulus heteroclitus*. J. Morphol. 163: 367–377.

GAULDIE, R.W. 1993. Continuous and discontinuous growth in the otolith of *Macruronus novaezelandiae* (Merlucciidae: Teleostei). J. Morphol. 216: 1–24.

GAULDIE, R.W. 1991. The morphology and periodic structures of the otolith of the chinook salmon (*Oncorhynchus tshawytscha*), and temperature-dependent variation in otolith microscopic growth increment width. Acta Zool. (Stockh.) 72: 159–179.

GAULDIE, R.W. 1987. The effect of surface sculpturing on the interpretation of opaque and hyaline zones in the orange roughy otolith. J. Appl. Ichthyol. 4: 140–146.

GAULDIE, R.W., D. DUNLOP AND J. TSE. 1986. The simultaneous occurrence of otoconia and otoliths in four teleost species. N.Z. J. Mar. Freshw. Res. 20: 81–92.

GRAHAM, M. 1929. Studies of age determination of fish. Part II. A survey of the literature. Fish. Invest., Ser. II, 11(3).

GREGORY, W.K. 1933. Fish skulls: a study of the evolution of natural mechanisms. Trans. Am. Phil. Soc., N.S. 23: 75–481.

HAN, V.C.F. 1964. Jackass fish (*Nemadactylus macropterus*). Rep. Dw. Fish. Oceanogr. C.S.I.R.O. Aust. 1963-64: 37–38.

JONES, C. 1986. Determining age of larval fish with the otolith increment technique. Fish. Bull., U.S. 84: 91–103.

KEIR, R.W. 1960. Answers to the questionnaire on age reading. ICNAF mimeographed document serial No. 714 (D.C. 2), document No. 4, 67 pp.

KELLEY, D.F. 1988. Age determination in bass and assessment of growth and year-class strength. J. Mar. Biol. Assoc., U.K. 68: 179–214.

KELLY, G.E., AND A.M. BARKER. 1961. Observations on the behaviour, growth and migration of redfish at Eastport, Maine. Rapp. P.-V. Cons. Perm. Int. Explor. Mer, 150: 263–275.

KOHLER, A.C. 1964. 1963 ICNAF cod otolith exchange. ICNAF Res. Bull. 1: 22–27.

LEE, R.M. 1920. A review of the methods of age and growth determination in fishes by means of scales. Fish. Invest., Series II, 4(2), 33 pp.

MAY, A.W. 1965. The validity of otolith ages of southern Grand Bank cod. ICNAF Res. Bull. 2: 19–24.

MCFARLANE, G.A., AND R.J. BEAMISH. 1987. Validation of the dorsal spine method of age determination for spiny dogfish. p. 287–300. *In* R.C. Summerfelt and G.E. Hall [ed.] Age and Growth of Fish, Iowa State University Press, Ames, Iowa.

MUGIYA, Y., AND T. UCHIMURA. 1989. Otolith resorption caused by anaerobic stress in the goldfish, *Carassius auratus*. J. Fish. Biol. 35: 813–818.

O'GORMAN, R., D.H. BARWICK, AND C.A. BOWEN. 1987. Discrepancies between ages determined from scales and otoliths for alewives from the Great Lakes. p. 203–210. *In* R.C. Summerfelt and G.E. Hall [ed.] Age and Growth of Fish, Iowa State University Press, Ames, Iowa.

PARKER, T.J. 1895. A course of instruction in zootomy. McMillan and Co., London. 397 pp.

PETERSEN, G.G.J. 1892. Fiskens biologiske forhold i Holboek Fjord, 1890–91. Beretning fra de Danske Biologiske station for 1890 (91), 1: 121–183.

PINHORN, A.T. 1969. Fishery and biology of Atlantic cod (*Gadus morhua*) of the southwest coast of Newfoundland. J. Fish. Res. Board. Can. 26: 3133–3164.

POPPER, K. 1983. Realism and the aim of science. Rowan and Littlefield, Toronto.

RADTKE, R.L., M.L. FINE AND J. BELL. 1985. Somatic and otolithic growth in the oyster toadfish (*Opsanus tau* L.). J. Exp. Mar. Biol. Ecol. 90: 259–275.

RALSTON, S. AND H.A. WILLIAMS. 1988. Numerical integration of daily growth increments: an efficient means of ageing tropical fishes for stock assessment. Fish. Bull. U.S. 87: 1–16.

SMITH, D.C. 1983. Annual total mortality and population structure of Jackass Morwong (*Nemadactylus macropterus* Bloch & Schneider) in Eastern Australian waters. Aust. J. Mar. Freshwater Res. 34: 253–260.

SMITH, D.C. 1982. Age and growth of Jackass Morwong (*Nemadactylus macropterus* Bloch & Schneider) in eastern Australian water. Aust. J. Mar. Freshwater Res. 33: 245–253.

SMITH, M.K., AND E. KOSTLAN. 1991. Estimates of age and growth of Ehu *Etelis carbunculus* in four regions of the Pacific from density of daily increments in otoliths. Fish. Bull. U.S. 89: 461–472.

TONG, L.J., AND C.M. VOOREN. 1972. The biology of the New Zealand tarakihi, *Cheilodactylus macropterus* (Bloch & Schneider). N.Z. Min. Agric. Fish. Fish. Res. Bull. No. 6.

VOOREN, C.M. 1977. Growth and mortality of tarakihi (Pisces: Cheilodactylidae) in lightly exploited populations. N.Z.J. Mar. Freshwater Res. 11: 1–22.

VOOREN, C.M. 1975. Nursery grounds of tarakihi (Teleostei: Cheilodactylidae) around New Zealand. N.Z.J. Mar. Freshwater Res. 9: 121–158.

VOOREN, C.M. 1973. The population dynamics of the New Zealand tarakihi, *Cheilodactylus macropterus* (Bloch & Schneider) and changes due to fishing and exploration. In Oceanography of the South Pacific 1972. [Compiled by R. Fraser.] pp. 493–502. (N.Z. National Commission for UNESCO: Wellington).

VOOREN, C.M. 1972. Post larvae and juveniles of the tarakihi (Teleostei: Cheilodactylidae) in New Zealand. N.Z.J. Mar. Freshwater Res. 6: 602–618.

WANKOWSKI, J.W.J., E. HYDUKE, AND S. WILLIAMS. 1988. Population age structure and mortality rates of Jackass Morwong (*Nemadactylus macropterus*) in Eastern Bass Strait, Australia. Fisheries Res. 6: 317–335.

WESTRHEIM, S.J. 1973. Age determination and growth of Pacific Ocean perch (*Sebastes alutus*) in the northeast Pacific Ocean. J. Fish. Res. Board Can. 30: 235–247.

Local Fisheries Co-management: A Review of International Experiences and Their Implications for Salmon Management in British Columbia

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The theory and practice of community-based self-management and government–community co-management is examined in terms of the potential of these management systems to address some of the major biological, economic, and political problems of the salmon fishery of British Columbia, Canada. Particular attention is given to government–multiparty arrangements that integrate the concerns of multiple interests, while recognizing the special rights of aboriginal communities. Elements identified as key to the success of various arrangements include: (1) logistical arrangements, such as clear boundaries, membership criteria, interception agreements, and management-unit sizes appropriate to the abundance of natural and human resources; (2) cost-sharing arrangements, such as local cost recovery and local volunteerism; (3) power-sharing arrangements through checks and balances between local multiparty boards, a provincial board, and the Department of Fisheries and Oceans. The processes engendering social learning, through which government and local bodies could move toward such regimes, are discussed through a review of relevant literature on interorganizational conflict resolution, public policy, and organizational learning. Many of the elements of success of both arrangements and processes are likely to apply to a broad range of fisheries co-management situations.

On examine en principe et en pratique l'autogestion par des communautés et la cogestion assurée par le gouvernement et les communautés, au regard du potentiel qu'ont ces modes de gestion de régler certains grands problèmes biologiques, économiques et politiques qui sont associés à la pêche au saumon en Colombie-Britannique. On accorde une attention particulière aux ententes entre gouvernements et des intérêts multiples, ententes qui assurent l'intégration des préoccupations de ces derniers et qui font place aux droits particuliers des communautés autochtones. Voici quelques-uns des éléments jugés essentiels au succès des différentes ententes : (1) des ententes sur des aspects logistiques comme la délimitation de frontières nettes, des critères d'appartenance, des ententes sur les droits d'interception et la grandeur des unités de gestion qu'on juge être appropriées à l'abondance des ressources naturelles et humaines; (2) des ententes à partage de coûts, par exemple, où il est prévu une récupération des coûts au niveau local et qui font place au bénévolat; (3) des ententes de partage des pouvoirs qui incorporent des éléments d'équilibre des pouvoirs entre les commissions locales multilatérales, une commission provinciale et le ministère des Pêches et des Océans. On se penche sur les processus à l'origine d'un apprentissage social, et qui pourraient permettre au gouvernement et aux organismes locaux de faire la transition vers de tels régimes, en examinant des documents appropriés sur la résolution des conflits entre organisations, sur les politiques gouvernementales ainsi que sur l'apprentissage organisationnel. Bon nombre des éléments qui ont conduit au succès de pareilles ententes et processus devraient pouvoir s'appliquer à toute une série de situations où il est question de cogestion des pêches.

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British Columbia's salmon fisheries face a number of biological, economic, and political problems for which no immediate solutions are apparent under current arrangements. Some aspects of these problems, on the one hand, seem universal and unavoidable, and would look remarkably familiar to scholars and managers who have worked with fisheries around the world. On the other hand, a growing literature on local management systems on every continent suggests that solutions to other aspects of these problems are very old, and have been reinvented independently by both dispersed pre-industrial societies and by encapsulated societies within modern nation states. This literature appears in biology (Johannes 1978, 1981), human ecology (Berkes 1981), cultural ecology (Acheson 1975; McCay and Acheson 1987; Pinkerton 1988; McGoodwin 1990; Albrecht 1990; Dyer and McGoodwin 1994), sociology (Jentoft and Kristoffersen 1989), economics (Bromley 1989), geography (Klee 1980, Ruddle and Akimichi 1984), history (McEvoy 1986), and political science (Ostrom 1990). Through greater interdisciplinary communication, the answers these local systems appear to offer are becoming better known, catalogued, and integrated into a general theory of self-management and co-management (Pinkerton 1989, 1991, 1992, 1994a, 1994b; Berkes at al. 1991; Smith 1994).

This discussion reviews a few key examples from the large literature on self-management, the much smaller literature on government–community co-management, and the sparse literature on multiparty co-management, as preparation

for considering what implications these systems might have for salmon management in British Columbia. Because these institutions of power sharing have biological, economic, and political implications, it is helpful to first revisit these aspects of key problems facing today's salmon managers and fishers in British Columbia. We may then consider how alternative systems might ameliorate these problems.

Biological Problems

Salmon habitat loss continues to occur through water diversion, dam construction, mining, industrial pollution, real estate development, inadequate regulation of logging practices and agricultural runoff, and uncoordinated land-use planning relative to watersheds as a whole. Many of these potentially habitat-destroying activities will become more intense as population increases.

Each Pacific salmon species may be subdivided into stocks, generally considered separate interbreeding populations, each adapted to the specific environmental conditions of its natal stream (Nehlsen et al. 1991). The loss of biodiversity both through the extinction of stocks and the steady erosion of smaller stocks has particularly alarmed scientists and residents of communities formerly dependent on them. Even though aggregate salmon harvests and aggregate escapements have increased in the last decade, these are based on fewer and fewer stocks, mostly sockeye (*Oncorhynchus nerka*) and pink salmon (*O. gorbuscha*) stocks and often enhanced ones (Northcote and Atagi 1994). For example, 90% of the sockeye biomass comes from fewer than 10% of sockeye stocks. The other 90% receive relatively little management attention (K. Hyatt, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC V9R 5K6, personal communication) One publication on South Coast stocks predicts that 35% of current stocks will be lost over the next 40 yr if harvest patterns remain unchanged (DFO 1988). Riddell (1993) notes that one third of the salmon populations known since the 1950s in the Strait of Georgia have been lost or have decreased to such low numbers that spawners are not consistently monitored. Many attempts to reintroduce salmon into areas where stocks have become extinct have failed, and it is not yet understood why successes occur in other cases (Withler 1982).

Dependence on fewer stocks is risky for many reasons (Walters 1988). Global warming and increased pollution, for example, could mean that stocks that are abundant now may disappear in the future, e.g., some Fraser River sockeye stocks, which are at the southern limit of their range (Henderson et al. 1992; Gilhousen 1990; Levy 1992; Groot et al. 1992). Conserving more stocks increases the chances that a number of productive ones will survive future changes and that annual cyclic variability in abundance will not occur in all stocks simultaneously.

One reason more stock-specific management is difficult to achieve is that the data gathering and analysis of the productivity of individual stocks, which provide the most critical information for managing salmon, are inadequate or insufficiently tied to aggregate management goals in most cases (B. Riddell, Pacific Biological Station, Department of Fisheries and Oceans, Nanaimo, BC V9R 5K6, personal communication). In many areas, stock-specific management is also hampered by the lack of in-season data on the timing and relative abundance of different stocks, and the fact that escapement targets can be "little more than educated guesses" without data on habitat status (DFO 1991). While biologists in the Department of Fisheries and Oceans (DFO) management system understand that there will never be enough resources to document all the stocks, many feel that far too many important and irreversible losses are being tolerated.

DFO is the centralized federal agency whose mandate is to collect and analyse data, administer licences, regulate harvest and habitat protection, and negotiate international agreements relating to salmon management. Important experiments by DFO have already suggested some promising directions for improvement, e.g., a planning framework for documenting salmon stock status in British Columbia, for generating options for improved management, and for piloting management innovations (DFO 1988, 1991, 1993a). This effort has been hampered so far by limited resources, and limited process development. DFO has also incorporated university-based research in a 15-yr adaptive management experiment to identify optimum escapement for sockeye in Rivers Inlet. Because the initial experiment did not produce clear results, the scientists compromised with local fishers, who supported a continued experiment involving harvest restraints only during abundant years (Walters et al. 1993).

Following these hopeful directions and other DFO discussions (Greer 1993), this discussion will review cases where local involvement in data collection, analysis, and monitoring of stocks and/or habitat is more extensive, long term, or institutionalized, and has already made significant contributions to the resolution of the types of biological problems described above.

Economic Problems.

Many B.C. fishers are no better off today than they were 10 yr ago, despite overall increased salmon abundance in British Columbia. As DFO brings in tighter regulations (shorter fishing times and more restricted areas) to protect weaker stocks, or to comply with the Pacific Salmon Treaty, fishing opportunities become shorter and shorter. As a result of this and other factors, fishers continue to invest in bigger and faster boats and more efficient gear to travel quickly to distant openings and to take full advantage of short openings. Fuel, vessel, and gear costs rise, even if salmon prices do not.

Increasing competition among salmon-producing nations for salmon markets is already exerting downward pressure on prices. For example, increased Norwegian and Chilean production of farmed salmon affects the top end of the market (Knapp 1992). At the middle and bottom end, Japanese–Russian joint ventures in production, processing, and marketing of sockeye, chum (*O. keta*), and pink salmon suggests that another cycle of price competition is likely to occur in the future (Kravanja 1992, Akaha 1993). Governmental management costs also continue to rise (Pearse 1982; F.E.A. Wood, Department of Fisheries and Oceans, 555 West Hastings, Vancouver, BC V6B 5G3, personal communication) while governmental budgets shrink.

In other words, the basic economic conditions of fishing continue to cut deeply into the benefits fishers and fishing-dependent communities might otherwise enjoy from the resource.

Examples presented in this discussion will suggest that both the cost of management and the cost of fishing could be lowered through a greater localization of management and greater participation of local communities or organizations in management.

Political Problems

Three types of political conflict have plagued the salmon fishery. The first is the longstanding demand from those involved in all sectors of the fishery to be far more involved in decisions that affect them. The Minister of Fisheries has broad discretionary and policy-making powers under the Fisheries Act. Although there are advisory processes at various levels, the sharing of power, data, and initiative in these is limited: agendas may be set elsewhere and recommendations may be ignored.

In most cases, fishers have little confidence that future ministerial policy will take their management concerns and their investments into account. Nor can they predict what basic values and goals will guide this policy. For example, fleet rationalization programs proposed or brought in by government over the last two decades have proved highly controversial, and have been seen as deliberately or inadvertantly dispossessing a particular group or sector without achieving the stated goals of the program (Pearse 1972; Hayward 1981; Marchak et al. 1987; Cruickshank 1991).

Exceptions to this pattern illustrate a more hopeful process for policy making, although they are rare, piecemeal, and not institutionalized. When the major commercial fishers' organization, the Commercial Fishing Industry Council, reached a coastwide agreement on salmon allocation among commercial gear sectors, the agreement was adopted by DFO as a working policy, at least for the moment (Brown 1993). DFO has begun to pilot more extensive consultative processes in key local areas as part of considering more stock specific management options (DFO 1991, 1993a; Walters et al. 1993). A key change in the latter type of process is an emphasis on stock management as opposed to fleet management, and an effort to involve fishers in in-depth discussions concerning long-term impacts of various fishing patterns on stocks. These beginnings are often attempts to transform regional advisory boards which can be narrow and nonaccountable lobbies for the status quo (Taylor 1993), to bodies more knowledgeable, participatory, and able to take a long-term view of trade-offs in management options. The long-range goal would be for all parties to be accountable to a management plan that they have agreed to implement. In the present situation, however, DFO may still unilaterally change the plan over the objections of all but the most powerful party.

The second type of conflict, namely between the long-established commercial fishery and the growing sport fishery, has intensified in recent years. Under the current policy framework, these two sectors tend to lobby Ottawa for the enlargement of their own access and the curtailment of the others' access, rather than working together to improve the resource or the management system. In the last few years, the commercial and recreational sectors have sometimes made common cause against allocations to aboriginal peoples, called "First Nations" in Canada.

The third type of conflict results from the Supreme Court of Canada's Sparrow decision and other recent cases affirming the rights of First Nations to a priority allocation of fish, as based in section 35 of the Constitution Act of 1982. In the context of this court decision, a major confrontation in Quebec between an aboriginal group and government (the "Oka crisis"), new public opinion polls, and constitutional negotiations, Ottawa adopted a 7-yr Aboriginal Fishing Strategy (AFS) in June 1992. One element of the strategy is to develop local co-management between Ottawa and First Nations (DFO 1993b).

First Nations are place-oriented peoples whose rights to fish are linked to continuous occupation and use of traditional local territories; these areas are usually within natural boundaries such as watershed ecosystems. Therefore, new co-management agreements developed between Ottawa and individual First Nations will involve a search for new local salmon management regimes to supplement and complement the work of province-wide regimes.

What are the implications of this for nonaboriginal commercial and sport fisheries? What opportunities exist for the peaceful coexistence of commercial and sport fisheries with expanded aboriginal fisheries? Could some degree of reorientation of the salmon fishery around local areas address the longstanding biological and economic problems of the fishery, as well as the new and old political conflicts?

This paper contributes to a more general discussion of the concept of fisheries co-management by reviewing international experiences with fisheries self-management and co-management. Particular attention is paid to multiparty examples, because the question of how First Nations can work together with existing commercial and recreational fisheries is a major concern.

Innovative answers to this question now appear possible because of new research in the social sciences regarding local institutions that can protect both ecosystems and the common property access privileges and/or rights of established fishers. These local institutions perform varying degrees of self-management and management in cooperation with government. In addition, this research calls into question the wisdom of government's conventional role as a manager who involves interested parties in a merely advisory capacity. Therefore, this paper also briefly reviews new models through which watershed-based groups build a collaborative process and/or public managers stimulate policy discussion among diverse parties by organizing and supporting a "social learning" process (Reich 1988; Dale 1989). In such an exercise, public values about a public resource can be refocused in the context of problems and dilemmas that must be faced. All of these approaches taken together form part of the answer to the problems of today's salmon management.

This paper first briefly considers the theory behind an alternative approach to fisheries management. The theory is then illustrated through international examples of community-based self-management by single groups, joint community–government management, or co-management between government agencies and single groups and joint community–government management in which multiple parties and their communities are involved. The discussion of these examples is then used to identify factors in the successful development of local co-management regimes. What does it take to have a successful institutional arrangement? Finally, the constructive role that collaborators and government can play in this process is considered.

Institutional Solutions to the Management of Common Pool Resources: A Brief Theoretical Overview

In parts of Europe, a privileged gentry still exercises private ownership and control of salmon streams (Netboy 1968, 1973; McCay 1989). In contrast, most North Americans of

European origin chose to manage salmon as a public and not a private good. How the public good is defined and managed is an issue of public policy. The definition of the public good has been heavily influenced by the bioeconomic paradigm sometimes described as "the tragedy of the commons," after the discussion by Hardin (1968). This paradigm asserts that common pool[1] resources, such as fish, will inevitably be destroyed by the users unless they are either privatized or very tightly regulated by the state. The privatization route has been largely rejected as unpalatable to North Americans, at least for salmon, and is, therefore, not discussed here.[2] Scrupulous state regulation has been seen as the way to protect a resource which is theoretically owned by all Canadians.

As noted above, state regulation alone has not solved important biological, economic, and political problems. Some of these problems have been solved or greatly ameliorated, however, by community-based forms of management, often working in tandem with state regulation. Hardin and early common pool theorists did not consider these forms of collective management viable. However, community-based management regimes display a common pattern, and are based on some of the same fundamental understandings as that of the bioeconomic model about what causes problems in the management of common pool resources. However, the "solution" is quite different.

Institutional economists and political theorists express this understanding in terms of two characteristics of a common pool resource such as fish: nonexcludability and subtractability (Feeny et al. 1990). A brief exploration of these two concepts will help explain how community-based regimes work.

(1) *Nonexcludability*. The physical nature of the resource (dispersed over hundreds of miles, present at unpredictable times, highly mobile, often invisible) means that limiting access to it is costly and in some cases impossible for governments acting alone. Limiting access can be a problem not only in the case of unlicenced fishers, but also for both the legal and illegal activities of licenced fishers. With thousands of licence holders in possession of highly sophisticated harvesting, refrigerating, and freezing technology, it is difficult for government to make sure that salmon are actually caught in the areas and times that are reported. (This applies to licenced and unlicenced recreational marine and all riverine fishers as well).

In addition, if thousands of fishers can harvest every local area of the coast to the same degree, there is little incentive for fishers to work on improving the productivity of specific areas. Moderate improvements in abundance might not be adequate to create a harvest "opening"; dramatic improvements might be wiped out in a single opening. In the latter case, benefits might be too widely shared to merit the continued hard work of those focused on particular areas.

Community-based regimes can be very effective at controlling access to the nearshore resource, and co-management regimes can greatly improve more distant water enforcement (Jentoft and Kristoffersen 1989; Pinkerton and Keitlah 1990). The community of members of locally based associations, First Nations, licence holders in a particular area, and/or simply knowledgeable community residents who know who can fish, add greatly to the capacity of locally based regimes to control the access of illegal fishers. Such local management systems can also devote much energy to management with some sense of security that they will enjoy some of the benefits. When a locally based regime has gained widespread community support and legitimacy, and there is a sense of ownership and responsibility for the resource among a known group of users, the entire community can become part of the eyes and ears of the local regime's enforcement (Pinkerton and Keitlah 1990). At the opposite end of the spectrum where these aspects of the management system are absent, one is more likely to find situations like the one documented by Kearney (1983) in Nova Scotia: Atlantic herring (*Clupea harengus*) quota holders and processors underreported deliveries by 40% during a crisis when DFO was blamed for resource problems and price failures.

(2) *Subtractability*. Common-pool resources are also problematical to manage because the harvesting activity of any individual subtracts from what is available for any other user. Average catch declines for every fisher when one more fisher enters the fishery or takes a bigger share.

Community-based regimes can limit the harvesters in any particular area to a number that can be sustained by the resource. The willingness of fishers to obey the rules appears to be related to their ability to monitor each others' activity to insure that everyone else is obeying the rules also (Ostrom 1990), as well as the degree of legitimacy of the regime, as mentioned above. When the fishing regulations are issued or validated by a locally based regime, in consultation with government and following principles of sustainable yield, and when a diversity of local people are knowledgeable about the conservation imperative of regulations and able to observe each others' behaviour, regulations will be more easily implemented.

It is important to note that this does not mean all community-based or artisanal fisheries automatically operate in this way. Cultural ecologists have observed that small-scale societies tended to develop stable human–resource

[1]The term "common pool" is used to distinguish those natural resources such as fish, wildlife, water, forests, rangelands, which by their very physical nature are difficult to manage under a simple regime such as private property. This is true partly because of the mobility, scale, or opportunities for secret use of the resource. The term "common pool" is used to distinguish the physical nature of the resource as opposed to the political regime or system of property rights through which it is managed. Common pool resources may be managed under at least four different types of political regimes or systems of property rights: open access (unregulated), private, state, community based, or combinations of these. The term "common property" is not used here, to avoid the possible confusion between the physical nature of common pool resources and the regume under which they are managed (Ciriacy-Wanthrup and Bishop 1975).

[2]Individual transferrable quotas (ITQs) as a form of privatization have been advocated for certain fisheries in British Columbia by some economists and opposed by others. ITQs have been implemented for halibut in British Columbia with the cooperation of current licence holders whose licences became far more valuable under the new policy. ITQs have been vigorously and consistently opposed by industry leaders and by crew and rental skippers whose method of payment has been transformed from an established share system to a lower wage (Cruickshank 1991). ITQs do extend the fishing season and market availability in fresh form for fish accessible for long periods, such as halibut. This advantage would not apply to the short availability and seasonal fluctuations of salmon. For these and other reasons, ITQs are not discussed here.

relationships in the first place only when there was a long period of adaptation, during which a community could learn by trial and error to live within the carrying capacity of the local environment (Lee and DeVore 1968). In this process, the resource must be somewhat "forgiving" so that people can learn how to limit their access appropriately before they do permanent damage to the resource (Pinkerton 1994a). Self-managing regimes survive in modern times only if they can continue to discipline their own members, and protect the resource from overuse by "outsiders" operating under a different system. Because of the difficulty of achieving this under conditions of resource competition in modern times, self-managing regimes tend to survive in more isolated areas, or in areas where the state has not been able to play an active role (e.g., Stoffle et al. 1994). More frequently, self-managing systems have either collapsed under pressure or evolved into some form of co-management (Pinkerton 1989, 1994a). In the ensuing discussion of self-management and co-management, it is useful to conceptualize co-management as 8 potential points on a 10-point continuum between state management (at point 1) and self-management (at point 10). In this discussion, self-management will be useful more as a conceptual tool than as a description of an operating system. This discussion also uses the term "comunity-based regime" to refer to forms of co-management that have an important component of self-management.

Common pool theorists, (Ciriacy-Wanthrup and Bishop 1975, Feeny at al. 1990) have pointed out that the type of resource regime or system of property rights under which a common-pool resource is managed has a great deal to do with success in dealing with the nonexcludability and subtractability issues. Resources managed actually or virtually as open access (no regulation) have usually collapsed tragically, as Hardin (1968) predicted in the original paradigm.

Resources managed by state regulation sometimes collapse because of inability to sufficiently exclude or to regulate the activities of users, as mentioned above. State management may also be vulnerable to political processes or government failures, which lead to suboptimal management or complete elimination of the resource (Feeny at al. 1990). The widely documented disappearance of aquatic species around the globe, related to both overfishing and failure to protect habitat, does not provide reassurance that most nation states are able to manage effectively (IDRC 1991; Larkin 1991; Nehlsen et al. 1991; Pitt 1993).

Resources managed under communal or community-based tenures (or mixed community–state tenures) were not considered by the original "tragedy of the commons" paradigm, although these are common throughout the world both as traditional regimes that operate without interference, and as mixed regimes that operate informally or formally under contractual arrangements with state governments (Martin 1989, 1992). A key difference in community-based regimes is that information about harvesting and habitat-destroying activities tends to be widely shared and mutually monitored by community members, i.e., there are no secret "free riders" disobeying harvesting regulations. Habitat destroyers will be subject to more concerted political pressure.

These regimes thus operate on a fundamentally different dynamic than the one imagined by Hardin, in which each individual user acted alone, in competition with, and in isolation from, other users. Hardin and his followers used the theoretical "prisoners' dilemma" situation to predict that individuals under state management would act out of a narrow individual self-interest.[3] A key feature of the prisoner's dilemma is the lack of information each harvester has about the intentions of the other harvesters, and the inability to affect the behavior of the other harvester. In a community-based regime, the "prisoners" or individual harvesters do not act out of ignorance or impotence regarding what other harvesters will do, but are part of the system that monitors and forces compliance on other community members.

Shared information creates opportunities for local groups or state–local bodies to create rules for self-regulation and to enforce the rules. Under appropriate conditions, as discussed above and below, such groups can be successful at dealing with the nonexcludability and subtractability issues. Furthermore, community-based groups in this situation can become proactive, rather than merely reactive, educating their members about resource problems and taking responsibility for solving these problems (Osborne and Gaebler 1992). While it would be a mistake to consider the community-based aspect of co-management an automatic panacea for the array of fisheries management problems, it would be equally unfortunate not to take advantage of the management benefits available under community or mixed community–government arrangements, particularly at a time when DFO resources for management are being cut back.

What is needed is a framework for analyzing the key elements of successful self-management systems, especially as incorporated into a co-management regime. The next section will begin to construct this framework through two illustrative examples.

Experiences with Community-Based Management and Self-Management

The two examples below illustrate some of the general characteristics of traditional self-management regimes. Such regimes tend to involve culturally distinctive methods of regulating access to resources, sustainable yield harvesting strategies, habitat protection, and social systems of production, distribution, and consumption that support sustained yield in balance with other resource uses. The management system is reinforced by systems of language, ritual, and spiritual beliefs that shape the interpretation of environmental experience and knowledge. Self-management also includes the notion of the community as a corporate group linked to specific lands and marine areas in a relationship which persists over generations. The corporate group has the ability to exclude, regulate, or monitor the impact of non-corporate users on local resources.

(1) Small community management of inshore and nearshore areas in Lake Titicaca, Peru. Comparing 151 fishing communities along the Peruvian shore of Lake Titicaca in the size and type of their locally controlled territories, LeVieil and Orlove (1990) noted three common characteristics. (1) Communities were able, despite government opposition,

[3]In this hypothetical model, two soldiers out of uniform are captured by the enemy and interrogated separately, without being told whether the other prisoner has confessed or not. The model predicts that each prisoner will "defect" in exchange for a reduced punishment, through fear that the other prisoner will do the same. The parallel is then drawn with the harvesting of resources such as fisheries, in which each harvester will overharvest, for fear that nobody will effectively prevent others from doing it.

to assert territorial control (exclude outsiders and regulate insiders) over their local areas, averaging 6 km of shoreline and 30 km^2 of aquatic zone; outsiders are repelled by threats or by destruction of gear. (2) The type of control depended on the costs and benefits of defending the territory: the surveillance, defense, and retaliation costs increased with the distance offshore, so that inshore and nearshore territories were defended more rigorously; offshore areas were defended only periodically, when schools of fish passed through. In offshore areas, outside fishermen recognized the informal local rights by giving the local community a small portion of the catch or comparable commodities. These arrangements were less costly and more beneficial than strict policing of offshore areas. (3) Fishermen received more of the benefits from territorial defense of fishing grounds and also paid more of the costs in time and risks associated with defending the territory than did other community members.

(2) Reef Management by Clan Chiefs in the South Pacific (Johannes 1978, 1981, 1988; Klee 1980; Ruddle and Johannes 1985; Iwakiri 1983; Carrier and Carrier 1989). These are some of the most thoroughly documented cases of self-management. Johannes' now classic *Words of the Lagoon* (1981) records 16 mo of fieldwork on local fishers' knowledge and management of reef species, during which he fished with and was tutored by the local fishers. Johannes documents not only Pacific islanders' encyclopedic knowledge of fish behaviour, but also the sociopolitical measures taken to regulate land, water, and resource use and achieve conservation in these complex and fragile ecosystems.

Johannes (1978, 1988) later extended his studies to generalize about the management measures used in many parts of Oceania long before they were considered in western management. These included limited entry, closed seasons, permanently protected reserves, size and area restrictions, gear restrictions, restrictions on taking eggs, allowing for escapement, holding excess catch live in pens until needed, and taboos on eating certain species. These measures were enforced through a system of marine tenure similar to the one described for Lake Titicaca, except that fishing rights were controlled by clan chiefs for the benefit of the municipality. Outsiders who had failed to ask permission to fish in the local territory (and offer a portion of the catch) were fined through their chief.

Experiences with Joint Community–Government Management or Co-management

Many traditional management systems, such as the South Pacific example, have weakened or collapsed under political domination or pre-emptive fisheries by colonial powers or national governments. In some cases, the authority of chiefs or the local group to regulate a local fishery was eroded directly by the activities of more powerful outsiders. In such cases, local group members might choose to respond to external markets and no longer be subject to local control. Missionaries sometimes directly attacked the ideological basis for traditional management through attempts to discredit the cultural belief system. It is, therefore, important to consider the two following examples in which, instead of collapsing, a local management system has been incorporated into a modern state, and allowed to operate virtually unchanged. These examples illustrate how the essential features of the traditional systems function in a modern context.

(1) Village multisector co-operatives in Japan (Commitini 1966; Ruddle and Akimichi 1984; Ruddle 1989; Short 1989). The Japanese system of communal sea tenure has remained intact from traditional to modern times. It has the same legal status as communal land tenure. Based on an ancient system of clan ownership and control of the local marine area, sea tenure was incorporated into Japanese law in 1901 (updated in 1949) through what had by then evolved into local village-based fishing co-operatives. All coastal waters except port areas and industrial zones are owned by fishing co-operative associations or federations thereof. All locally resident fishers who are members of their local co-operative and work 90–120 d a year (depending on the cooperative) enjoy rights to fish particular species in their area (Ruddle 1989). In addition, the prefectural government (equivalent to a county or regional district) grants a very limited number of licences to large-scale set-net locations for herring and salmon fisheries if there are appropriate locations for them. Small set nets for these species are allocated and managed by the co-operative. In some co-operatives, some waters may be reserved for the use of a particular fishery, such as set nets or octopus holes, which are sometimes inherited; highly productive areas may be allocated by lottery. Fishing rights cannot be loaned, rented, mortgaged, or transferred by individuals. Any infringement or loss of these rights by the co-operative must involve compensation to it. (In one industrializing area, industry compensated fishers in the cooperative for the destruction of their fisheries (Befu 1980). The frequent failure to protect habitat from industrial pollution is the one serious shortcoming (Wigen 1989).

The local co-operatives manage their areas by implementing and enforcing national fishery laws and regulations (e.g., that harvest levels be sustainable), supplemented or complemented by those made locally. Local regulations involve conservation measures and membership. The co-operative meets with prefectural and national governmental offices to obtain approval of its regulations and to negotiate licencing and other rights. Although ultimate control of a co-operative rests with a general membership assembly, daily business is conducted by an elected executive committee, including one or two paid administrators. These work with sectoral fishing groups, which make recommendations about how to implement regulations in their particular fishery. Some co-operatives with highly diversified fishing sectors might more properly be considered multiparty co-management. However, because analysts believe the high level of compliance results more from informal social pressure than from formal enforcement (Kalland 1981), this classification is not used here. Consensus decision making must meet the goal of accommodating in some way the interests of all concerned. The Japanese local management system has been remarkably stable over time, and has been able to accommodate highly productive aquacultural innovations in species such as geoduck and scallops.

(2) Community enforcement of individual access: Maine lobster. The lobster "fiefdoms" of the state of Maine constitute an informal community-based marine tenure system that has recently gained some degree of state support (Acheson 1975, 1988; Bowles and Bowles 1989). Lobstering communities in the more isolated outports of Maine are more successful than less isolated areas at defending their territories through a system of limiting access to community members. Community members constitute a "harbour gang"

that not only limits to gang members those who can claim a local lobster territory, but also enforces the rules about respecting other members' territories on all lobster fishers. Although, technically, anyone can buy a lobster licence, lobster fishers who defy local customary rules do not last long. Destruction of gear is the chief method used to censure those who intrude on an individual lobster fisher's "fief" or those who fish the area without participating in the local gang that allocates the fiefs. Fishers in the better defended outport areas enjoy a far higher catch per unit of effort, because the mean lobster size is much larger and they make fewer trips to collect them, having exclusive use of their territory.

The lobster example illustrates a common feature of forms of self-management regarding more stationary resources such as shellfish. Management rights in these more predictable resources tend to be individually held, while in the same area rights over less predictable, mobile resources are communal. The same patterning of individual and communal rights is also found in Lake Titicaca and Japan as well as in other fisheries and in terrestial common pool resources (LeVeil and Orlove 1990; Netting 1976; Ostrom 1987).

However, in the Maine lobster case as in other self-managing systems, individual rights exist in the context of local community rights, and are upheld by them. In recent years, harbour gangs have produced regulations on closed seasons and trap limits, which they have persuaded the state to adopt. Harbour gangs favoured trap limits to be set at different levels appropriate to each region. Limits were brought in by government based on specific agreements with lobster licence holders in particular areas. The existence of state regulations lowers the monitoring and enforcement costs of harbour gangs, and serves as an important support to the local informal management system. A large degree of self-regulation by the harbour gangs likewise lowers the cost of enforcement by the state.

Experiences with Multiparty Co-management

(1) Self-regulating, multistakeholder watershed-based users' associations: southern California groundwater users (Blomquist 1992; Ostrom 1990). Self-management and co-management systems can also arise in the absence of a shared tradition, social system, or religious system, as illustrated by an example outside the fishery, used here because of its longevity and institutional evolution. A compelling economic need to sustainably manage scarce groundwater forced local users in southern California in the 1940s to work together. Basin by basin, watershed by watershed, they crafted self-governing institutional structures that have proved highly successful to the present day. These are based on the ability of users to monitor each others' use and have mutual accountability through a hired co-ordinator, the watermaster.

Groundwater users in the Raymond Basin near Pasadena were the first to innovate; neighbouring basin residents learned from them and followed suit. Raymond Basin faced salt water intrusion into underground aquifers because of competitive and nonsustainable use of groundwater, high costs of litigation among competing users, and high costs of importing water if the basin were irreparably damaged by overuse. By reaching out-of-court agreements and jointly hiring a watermaster to monitor and enforce the rules they agreed to, the many small individual users and the larger water companies achieved a guaranteed and sustainable supply of water at a much lower cost.

Key to the successful establishment of self-managing water users associations were public discussions among users, joint commissioning of studies, and considering options, e.g., how individuals were likely to behave in response to various strategies, and to what behaviours individuals were willing to commit themselves. Once these commitments were made, the costs of mutual monitoring and enforcement could be kept quite low. The watermaster was directly accountable to the users, and a user who practiced noncompliance would have a great deal to lose. (It is important to consider what fisheries offer opportunities to keep monitoring costs low by mutual monitoring, and which offer greater opportunities to cheat. The salmon fishery falls into both categories at different times and places and might require a diversified monitoring system in which mutual monitoring could play and important, but perhaps not exclusive, role.)

During the first 40 yr, the Southern District office of the California Department of Water Resources served as the Raymond Basin watermaster, and the water users paid only 50% of the management costs. In 1984, the Raymond Basin Advisory Board, which had become increasingly active in seeking innovations and learning from activities in other basins, took over the administration and 100% of the costs of management. The management board, henceforth, comprised 10 water users' representatives and normally operated by consensus. It retained the services of the Department of Water Resources under contract for the preparation of annual reports and other support services, as required by the court, which retained jurisdiction to decide future controversies and to rule on alterations to institutional structure such as the above. Since 1984, the management board has attempted to manage the basin with more precision and to experiment with more flexible arrangements for water storage, credit spreading, and voluntary pumping pattern adjustments. As other water basins established their management structures, the water boards have formed networks that have exchanged and shared services. The management program has stopped the overdraft of water without ever having to use sanctions to enforce the injunction against overpumping in nearly a half century. The system has kept administrative costs low and shown considerable adaptibility to changed basin conditions and new ideas.

This example suggests that enormous differences in levels of investment and in technology used, such as exist among different gear types in the B.C. salmon fishery, need not be an impediment to achieving agreement. What was crucial was the public discussion and exploration of goals and possible strategies, the support of government in facilitating the acquisition of key information about the status of the resources and the risks involved in various strategies, and the role of the courts in ruling that each user must share equally in reducing use by 31%, regardless of seniority. These three conditions allowed users to move beyond a prisoners' dilemma situation in which each party acts out of solitary ignorance of both other parties' intentions and the state of the resource. Without access to critical information (such as information on particular stocks), and the ability to make informed decisions about long-term costs and benefits, First Nations and stakeholders are not likely to form the

alliances that lobby for reform and effective regulation. Without access to forums in which they can interact creatively with one another and commit themselves to mutual planning and monitoring, they will not form self-regulating associations.

Locally based management agreements that involve different gear groups, sectors, or differently situated parties can still be based on the same principles as the above arrangements, if they are area based or ecosystem based. Several variations on this theme are presented below. The first example is more regionally based than ecosystem or watershed based, and hence requires more time and effort without these natural unifying forces.

(2) A Fishers' regional enhancement association (Amend 1989; Pinkerton and Langdon 1987; Pinkerton 1994b). The Prince William Sound area of Alaska, and the fishing community of Cordova in particular, led the state in lobbying for legislation to permit the setting up of fisher-operated nonprofit associations that could borrow money from a revolving state fund to finance the construction and operation of hatcheries and other salmon-enhancement projects. Projects were developed by the fishers' associations and approved by the Alaska Department of Fish and Game. The "aquaculture" associations basically practice cost recovery enhancement in a manner that dedicates two thirds of the enhanced returns to the "common property" offshore fisheries by their own members, and one third to the association, which catches the fish terminally and sells it to support the activities of the association. (The association also develops regional enhancement plans for its area jointly with the state.)

There is only one association per fishing area, and association membership is limited to the fishers licenced to fish in Prince William Sound, one of six salmon fishing areas in Alaska. Alaska has a system of licence limitation by area, although fishers may own licences in more than one area and do not have to be resident in the area they fish. In practice, fishers tend to fish only one area per species. Those licenced to fish in a particular area, therefore, have an incentive to build up the runs in that area, and do not have to share the benefits with an Alaska-wide fleet which has done nothing for the local area. There are approximately 200 seines and 500 gillnets licenced to catch salmon in the Prince William Sound area.

The association was originally formed by majority vote of the fishers, who then elected a representative board to run the association and hire staff. The association in Prince William Sound is run by a board of 43 (of which 23 are fishers), which meets in January, June, and September. One third of the board is elected each year by all licenced commercial fishers in the area. An executive committee is elected by the full board every June, meets monthly or more often, and distributes minutes and a monthly newsletter to the other board members. The full board sets general policy and the executive committee implements it.

The association was so successful in the enhancement of pink salmon that its sales of one third of the returns enabled it to begin supporting management activities for which it took responsibility alongside the state. For example, the association developed a plan for the allocation of enhanced salmon between seiners and gillnetters, the two major gear groups involved which participated equally on its board. The association planners forced small groups of seiners and gillnetters to work together until they reached some working agreements on what would constitute fair allocation. These groups then reconvened in larger sessions and developed an area-wide allocation plan that had widespread support.

The association also developed a co-ordinated harvest management plan for wild and enhanced pink salmon stocks. As concern developed in the early 1990s about the potential impacts of a mixed stock fishery at one entrance to Prince William Sound, the association dedicated more money to tagging, tag recovery, and escapement counts; the entrance mixed-stock fishery was closed by 1993. In short, a fishers' association that began as a body dedicated to enhancement began to take on more and more harvest management responsibility as their successful operation became established.

Although the legislation required that a majority of board members be commercial fishers, the association boards were also required to represent fishing-dependent communities in the area, and Native corporations. This meant that the fishers' interests had to be tied to the interests of local communities, even if they did not live in the area. A sizeable minority of licencees in Prince William Sound in fact do not live in the area, and are thus less likely to attend all the board meetings and influence the association. Because of its ability to include the interests of both resident and nonresident users, the association offers a helpful model for a situation like the one in British Columbia, in which fishers may have long-term ties and dependencies on fishing areas distant from their residences.

Although local residence is an ideal condition for the development of community-based management, the Alaska model suggests that it is not necessary for all users to be resident as long as there is a core group of active residents involved in management who are accorded, at least informally, a special status based on stewardship. The community-based development that is likely to emerge from local enhancement activities (see below) may also encourage more of the stakeholders to relocate to the area.

(3) Watershed management by multiparty local committees (Pinkerton 1991; Dobyns 1992; Seiter 1993). While the Alaska model illustrates that regional multiparty salmon management is possible, a Washington watershed planning model illustrates how different interest groups can work together effectively on a smaller watershed level, especially with the benefit of some education and guidance from a co-ordinator. The model is especially useful as an illustration of effective mechanisms for achieving consensus, legitimacy, and volunteer contributions. It eventually evolved into a forum in which tribes operated as governments, but planned jointly and through consensus with other watershed interests. Their activities focused in this case on habitat protection rather than harvest management.

Because of increasing concern in Washington State about the pollution of Puget Sound, the Puget Sound Water Quality Authority was set up as a superagency in 1985. In 1987 the Authority initiated a nonpoint source pollution control program to stimulate local planning to implement statewide water-quality standards. The agency targeted key watersheds with valuable salmon runs or shellfish-rich estuaries; it asked counties to convene local representative committees (including tribes and nontribal commercial fishers) from watershed residents. Each county hired a co-ordinator to select a local planning committee, and to assist the committee in producing a water-quality plan. This plan set target community water-quality standards, and a method and

timetable for achieving these standards. A comparative study of nine such planning processes (Pinkerton 1991) suggested that, where there was an effective local co-ordinator who combined education about water quality (sources of pollution, known methods of reducing pollution) with creating dialogue and a problem-solving approach among members, the planning process was quite successful in creating a consensus about how to priorize and address pollution problems, and an action plan for implementation. The planning process was successful also because it operated within a clearly defined mandate which empowered the county to require compliance from other agencies, once the plan had gone through an approval process. Typical actions taken under the successful plans included (1) farmers fencing cattle out of salmon streams, resulting in lowered erosion and pollution; (2) septic tank upgrading, resulting in lower fecal coliform counts; (3) higher than state standards set for logging in the riparian zone, resulting in greater streambank stability, shading, and large woody debris to create pools; and (4) co-operative stream restoration or enhancement projects, beach clean ups, or safe toxic waste collection and disposal days, using community volunteer labour.

The combination of having a powerful mandate and achieving local consensus by a democratic process gave the plan a high level of legitimacy and support. This generated a local constituency that could apply pressure on local agencies to implement the plan, as well as existing regulations (currently unenforced). This latter process happened most notably when a corps of local volunteers was trained and organized to carry out projects in the watershed and to educate the rest of the community on water-quality problems and solutions. In terms of fish habitat protection in particular, the water-quality planning process at the local watershed level was a very effective tool in creating the political will and support to make habitat protection more effective. Volunteers reported violations and worked on major stream projects, including rechannelling to avoid major pollution sources.

Even more important, a plan on the watershed level can deal with diverse sources of nonpoint pollution in a comprehensive way. The regulatory framework of government agencies often does not permit the cumulative effects of many different sources of pollution, normally regulated by different agencies under different laws, to be dealt with in this way, and fish managers do not have the staff to cover it.

The processes begun by watershed-based water-quality planning were continued through a second process to solve water allocation disputes among multiple parties in one pilot watershed, the Dungeness–Quilcene on the Olympic Peninsula. In 1990 the statewide Chelan Agreement brought together tribes, agriculture, fisheries interests, recreation interests, power companies, other industrialists, and the affected state agencies. Their agreement in principle about how water could be equitably shared is in the process of being tested in this pilot project (and one other not discussed here).

The Dungeness–Quilcene watershed planning process is of interest to this discussion chiefly as a model for how tribal governments can fit into a government–multiparty process. This exercise occurred after the Centennial Accord had been signed, which recognized tribes as sovereign governments, and affirmed the government-to-government relationship between state government and tribal governments. In the Dungeness–Quilcene project, the Jamestown S'klallam Tribe acts as local government and co-ordinating agency, playing the role parallel to that played by the county in the water-quality planning process described above. Because several of the county commissioners in this watershed had also been previously involved in that successful water-quality planning project, their willingness for the tribe to be co-ordinating agency indicates a vote of confidence that the tribal interest is likely to coincide with the self-interest of people in the watershed as a whole. All the meetings are held on the reservations in tribal facilities.

For the other committee members, the original structure of decision making envisaged by the signatories to the Chelan Agreement, and suggested for the pilot projects, was not acceptable. According to this model, a decisive vote on the committee would have involved the agreement of all three governments (tribe, county, and state agencies that are led by Department of Ecology) plus three out of the five local interest groups representatives. The interest groups (recreation, fisheries, environmental, business, and agriculture) would not accept the possibility that "governments could gang up on us," and insisted that all decisions be made by consensus. This has been accepted for the present, with the proviso that the statewide decision-making method might be reconsidered if reasonable progress cannot be made with consensus. This model is important as an acknowledgement that, even if some parties have greater or prior rights, the interests of effective management require that they all work together.

(4) The importance of habitat as the overriding common interest (E.W. Pinkerton, unpublished data). These two watershed management models are key examples of the importance of habitat protection as a common focus for stakeholders who may be in conflict over other issues, such as allocation. Because these groups were initiated for the purpose of resource protection, and after major fisheries-allocation battles had been settled, it was easier for groups to focus on their more fundamental common interest in healthy habitat. This possibility has been realized at the watershed level among all stakeholders, but sport and commercial fishers have not joined habitat protection efforts at the state level in a significant way.[4] Partly as a result, the focus of the Washington Department of Fisheries on habitat has not been a strong priority, and support has been fragmented.

The tribal–nontribal allocation battles and the tribal–state power battles from 1970–1985 in Washington State (surrounding the 1974 *US versus Washington* case, its appeals and implementation) created a major distraction from habitat issues (Bruun 1982). Enormous effort was put into developing a salmon harvest co-management plan for Puget Sound, adopted by the state and tribes in 1984, in which the tribes and Washington Department of Fisheries shared power at each stage of planning (Cohen 1986, Pavel 1989). The tribes fought hard, and often successfully, for greater attention to management of individual stocks or stock groupings, especially after they had won a court ruling (*Hoh vs. Baldrige*

[4]Sport and commercial fishers could participate at a statewide level through raising fish habitat protection issues at meetings of the Pacific Fisheries Managment Council or the Forest Practices Board or working with the tribes or environmental groups who have participated in the Timber, Fish, and Wildlife Agreement since 1986. Trout Unlimited, a sportfishers' group which has participated in the latter forum, and recently Washington Trout, are notable exceptions.

1981), which recognized the right of each tribe to force management attention to its own local fishery and its stocks. The tribes also took these battles to the Pacific Fisheries Management Council (involving ocean interceptions) and the Pacific Salmon Commission (involving international interceptions), but they had to compromise with their state comanagers and other parties more interested in mixed-stock fisheries (Pinkerton and Keitlah 1990). Some tribes also felt that they should not have to pay the entire price for past management shortcomings by being the only party without some mixed-stock fisheries or some dependence on hatchery stocks. These tensions eventually created a complex co-management system with a high level of data, co-operation, and sophistication in harvest management.

The tribal–state co-management system fared less well in habitat protection, even though a major effort was launched by tribes and environmental allies to reform logging practices in 1986 (Pinkerton 1992). By the time the loss or endangering of salmon stocks had been documented and related in a more systematic way to habitat loss (Nehlson et al. 1991; Bisson et al. 1992), the trend had become difficult to arrest or reverse. By 1993, all the coho salmon (*O. kisutch*) stocks in the Pacific Northwest were under petition by environmental groups for listing under the Endangered Species Act. By 1994, nine additional (non-coho) Puget Sound stocks were petitioned for listing by an association of employees of the Washington Department of Fisheries, and environmental groups were preparing even more petitions. An endangered species listing would force management agencies to terminate virtually all fisheries that could impact the listed species, causing a major disruption to normal fisheries. Greater concern about stock status caused the Pacific Fisheries Management Council to close ocean fisheries completely in 1994. Many scientists fear that cycles of low ocean survival and unfavourable climatic conditions (which have occurred before) are now so exacerbated by loss of habitat quantity and quality (a new condition) that a number of depressed stocks will not rebound when ocean survival improves (Lichatowich 1994; Seiler et al. 1992; Lawson 1993). There may indeed be, as Alverson and Larkin (1993) warn, "much more to worry about from habitat degradation than from overfishing."

Habitat is an overriding concern because fish as a common pool resource are always situated within another common pool resource, water: fish are a commons within a commons, as R. Bish notes (personal communication 1994). Competition for water use comes from actors far more powerful and threatening to fisheries than groups of fishers (Bruun 1982): hydro-electric companies, multinational aluminum and timber companies, heavy industrial polluters, real estate developers, and irrigators. There is little doubt that, in the end, nontribal commercial and sport fishers will have suffered as much or more from habitat loss than from sharing 50% of the resource with the tribes under the 1974 *U.S. versus Washington* decision. The tribes and all fishing-dependent communities have lost a great deal as well: Bill Smith, the first chairman of the Northwest Indian Fish Commission (the umbrella group which co-ordinates tribal fishing issues among tribes) notes that "50% of zero is zero", adding that "the cost of those battles in terms of lost humanity of people to people is terrible." Smith's insight is shared by many scientists who agree that the solutions to habitat protection will require nothing less than fundamental changes in human attitudes and social structures (Lawson 1993; Alverson and Larkin 1993).

The success of watershed planning exercises suggests that one of the most direct ways for habitat protection efforts to be effective is to unite fishers, and other watershed users and landowners, around specific watersheds where they can best see their common interests, and build the human relations that enable effective common action. To affect policy statewide, however, local efforts must be co-ordinated. Although local planning has involved some individual fishers, their involvement has not been extended to the state level. At the state level, the main habitat protection efforts in Washington have been led by statewide tribal and environmental umbrella groups, with little support from commercial and sport fishers. Therefore, much of the support that was forthcoming has focused on wildlife and endangered species protection.

Fishers would do well to take the lead in fisheries habitat protection; if environmentalists take the lead by default, they are likely to have different agendas than fishers in protecting salmon. Some agendas could potentially create even greater management difficulties than already exist. It is crucial for all fishing sectors to see their differences within this larger perspective. The Washington experience suggests that if fishers do not unite around their common interest in protecting habitat, they will not only lose major portions of the resource forever, but they will lose control of the habitat protection and biodiversity agenda. These will be major future issues in B.C. as population expands and as competing demands on water and fish habitat increase.

Conditions For Successful Co-management of Fisheries

The variety of models for multistakeholder co-management show that the problems addressed by locally based regimes may be approached in a number of ways. There is not one way, and there may not even be a best way. However, the features that are widely shared in the models suggest that they are good predictors of success. In addition, systems work better when they have multiple reasons to work: when they perform multiple functions and have multiple forms of support. The following sets of conditions are identified as the ones most key to successful arrangements. They should not be viewed as absolutes, but as cumulative elements which work in tandem with one another under the general principle: the more conditions apply, the better chance there is for success.

Success is more likely when certain logistical problems are solved:

(1) Clear boundaries. Incentives for local stewardship of areas are stronger when boundaries are clearly delineated. The ability to exclude at least some users from local areas creates a greater sense of ownership and responsibility among local users. There may be benefits to setting aside some areas for the exclusive use of groups that can easily defend and regulate them for specific purposes (or the boundaries could be enforced by government, with assistance from local bodies and citizens). Different boundaries could exist for different purposes. Clear boundaries were a feature of all seven examples discussed above.

(2) Clear criteria for membership or participation in local area management. Membership in local associations or in local watershed management boards, which could grow out of associations, need not be limited to local residents in the beginning, or perhaps ever, as illustrated in Prince William Sound. Local residents, both First Nations and non-native, could be considered to have the greatest stake, however, and thus have more influence on decisions. This would be especially true in areas where local residents have no alternative sources of employment and are particularly resource dependent. The greater role of local residents could be recognized either through the structure of representation on local management boards, through a co-ordinating agency role, some degree of veto power, or a host role as defined by some First Nations' customs. Clear membership criteria was a feature of all seven examples above.

(3) Management units of a scale appropriate to human resources and the ecology of any particular area. Examples showed that local stakeholders tend to protect and manage resources within their territory in proportion to the costs and benefits. Different size areas may be defended or managed in proportion to their proximity or their value during particular times, as evidenced particularly in Lake Titicaca and Maine. There are trade-offs between management units being small enough for easy monitoring by community members, and large enough for more comprehensive management of interacting sets of stocks.

Ideally, management units would be based on watersheds, combinations of watersheds, or coastal zones. They could include both exclusive and in-common areas for different users and purposes. For example, certain in-common areas could serve as buffers to equalize fishing opportunities when some areas have suffered relative scarcity, or when an unusually large run is concentrated in one area and a larger fleet is needed to harvest it. The appropriate scale for management units ideally would evolve from larger units to smaller units when and if the capacity and desire develops among local populations to manage smaller units.

Because the success of any local management system would depend vitally on the active support of local fishers and communities, these parties would be in the best position to propose and debate concrete options regarding scale. DFO, or others, could then use computer modeling to project the specifics of these options, and to offer other options for public consideration. Any attempt by government to authoritatively designate specific areas would undercut the benefits discussed in this paper. Any attempt by government to use area-based management as a method of fleet rationalization would also defeat the purpose. In fact, area-based boards would be better situated than government to decide what the optimum number of fishing units should eventually be in their area. They would be motivated to consider resource sustainability, effective management, and the full range of community economic benefits. All seven examples discussed above were based on a concept of scale which balanced natural and human resources in one or more critical ways.

(4) Clear interception agreements. Local groups will not have incentives to do management planning, enhancement, or habitat protection if all the benefits of their efforts are captured by intercepting parties. It is in the interests of interceptors to allow enough salmon to pass through to terminal areas so that terminal residents of these areas will continue their work in rebuilding and stewarding particular stocks. Interception agreements must be worked out among all parties, before any rational planning can occur. These agreements could help frame other management options, such as those accorded local enhancement or habitat improvement associations. Principles used in the Pacific Salmon Treaty could provide useful models for interception agreements. For example, percentages of interception could change on a sliding scale depending on stock abundance, so that basic minimum amounts are assured to terminal areas. The sooner these agreements could be reached, the sooner local management options could be considered. This discussion assumes that international negotiations over interceptions through the Pacific Salmon Commission would continue as before, with probably greater inclusion of local board representation. With the exception of Washington State, this condition was not applicable to the examples discussed above.

(5) Local all-stakeholder co-management boards. The fundamental building block for co-operative management would be a local board with members representing all interested parties. Boards could provide the forum for discussion of all local fisheries management questions, the development of plans, and the review of management actions. Interested parties who would otherwise be disruptive are best brought into the process, if they are local stakeholders with legitimate concerns for the fisheries. The process would be most effective if it were set up so that all parties had more to gain by participating and working to solve problems together than by not participating or by attempting to disrupt the process, or using the courts. This means that some accommodation would have to be made for their legitimate concerns. Also, government would have to respect the integrity of the process, resisting the pressure of those who might try to circumvent or abort the process. At the same time, parties that came together would have to commit themselves to addressing basic resource sustainability problems, rather than acting merely out of a narrow and short-term self-interest. This condition applied in some form to all the postindustrial examples, and in a general sense to the preindustrial ones.

(6) A co-ordinating role for a province-wide management board. An overall province-wide board could co-ordinate management through a coastwide stock management plan, dispute resolution between local boards, and major policy issues affecting the whole coast. For example, it could help select representatives to be included in the Pacific Salmon Commission negotiations. The provincial board would need to include representation from communities and regions alongside province-wide organizations that represent fishing organizations and tend to have a lower-mainland perspective. Part of the role of this board, however, would be to serve as a check and balance to the perspectives of local boards. This type of structure, or its equivalent, was present in the cases of Japan, the Washington State watershed planners, Prince William Sound, and arguably in the California groundwater users' case.

Success is more likely if some of the costs are assumed "in kind" (e.g., through contributed volunteer labour and materials) by local bodies, related to the assumption of certain responsibilities and the ability to generate revenue. This condition applied formally in the case of Washington State watershed planners, Prince William Sound, and California groundwater users; it applied informally in all the other cases.

(1) Cost recovery related to local management activities. Legislation could allow local management boards and local enhancement associations to capture some of the benefits from local production, including enhancement, habitat protection, and, even more important, better wild stock management. Revenue could be raised through fish sales, landing taxes, licence fees, etc. These funds could be used to support operations, including hiring biological staff, collecting data in a fashion co-ordinated with other areas and DFO, possibly organizing or hiring support or substitutes for enforcement officers or related community projects. It would be important for local bodies to have the ability to borrow funds and receive partial funding for a start-up period, especially related to the hiring of a co-ordinator to help various participants work together. Aspects of this condition were present in the case of Prince William Sound, Washington State watershed planners, Maine lobstermen, and California groundwater users.

(2) A local volunteer force. A corps of local volunteers could back up and enhance the activity of full-time professionals. A diverse local management board with community representation, or the co-ordinator of this board, could arrange training and direction for a local corps of volunteers who could serve in several roles. They could assist in gathering information from other community members and from direct observation and then communicating it to enforcement personnel on violations of local management plans, whether harvest-related or habitat-related. Working with a staff biologist, trained volunteers could also perform important harvest monitoring and habitat monitoring services. Such a force would also play a key role in raising community awareness and support for excellence in management, and even help to create an important political constituency for adequate public funding for all management activities. This kind of recruitment of the community is possible with a sense of local ownership and responsibility for the resource, and a greater awareness of how local economies are tied to a healthy and well-managed resource. In areas where traditional aboriginal management systems are in place, the local board could assist in co-ordinating the activities of volunteers with theirs. This condition was present in Washington State watershed planning, and to some degree in Prince William Sound. It existed by implication among California groundwater users, and in different forms in Japan, South Pacific, and Lake Titicaca.

Success is more likely when certain objective political conditions are met:

(1) A degree of local control. When local association or management boards are allowed to exercise a sizable measure of control in "doing things their way", it is easier for them to generate support, enthusiasm, volunteer energy, and the sense of stewardship that constitutes the most important contribution of local management. In this way, existing social and political capital (local knowledge, experience, expertise, credibility) in the communities can be used and developed. Of course, local control in modern times and in dealing with migratory resources must have checks and balances through region-wide and senior governmental institutions, including DFO and the courts. The key to effective functioning would be flexibility and adaptability, as was demonstrated in the Southern California groundwater users' case. This condition applied to all the cases, although local associations or communities differed in the degree to which they shared power with regional or state-level institutions.

(2) Clear legal definition of local powers. Local bodies will take themselves seriously and put out more effort when there is enabling legislation or clarification of rights sufficient to give local management boards confidence that their work will not be disrupted by outside forces (e.g., regulation of other water users, conflicting jurisdictions) or dismissed as irrelevant or trivial (ministerial whim). This condition applied in Prince William Sound, California, Washington State, Japan, and eventually to some extent in Maine.

The Importance of Social Learning

Success is more likely if a social learning process occurs among different stakeholders, such as occurred with the southern California groundwater users and the Washington watershed planners. Social learning occurs when parties learn to redefine situations in terms of what they can achieve collaboratively. This involves not only a restructuring of perception, but also a willingness to take actions co-operatively with parties previously considered unknown and/or untrustworthy. Social learning can occur at both the provincial or state level and at the regional or local watershed level.

The California groundwater users example demonstrates that social learning among parties and stakeholders can occur without major government involvement, although the court ruling on equitable use reduction was critical in forcing one challenger to comply. Conversely, the Washington example shows that government involvement can be a powerful catalyst, and that having a convenor with appropriate stature, be it the national government, state government, country government, or tribal government, is one way of gaining some of the impetus, financial resources, and logistical resources to spur a collaborative social learning process.

Whether or not government is involved, the key question is: by what process do parties get beyond the "prisoners' dilemma" and learn how to work together? Useful responses to this question are found in several literatures, including the organizational behaviour, interorganization behaviour, and organizational learning literature in sociology (e.g., Argyris and Schon 1978; Gray 1985, 1991; Brown 1991) and the public policy analysis literature in political science (e.g., Reich 1988).

The first type of literature was usefully summarized and applied by Kofinas and Griggs (1993), which Pinkerton et al. (1993) applied in modified form to multiparty watershed-based collaboration. The basic "rules" for building multiparty collaboration can be summarized as: (1) having a convenor perceived to have appropriate stature, power, and purpose; (2) obtaining adequate representation of all relevant sectors; (3) finding a locally accepted basis for the legitimacy of participants; (4) finding a style of facilitation appropriate to the local situation; (5) establishing a shared definition of the problem; (6) establishing clarity about expected outcomes; (7) undertaking joint tasks, such as information searches, and assigning tasks to subgroups; (8) articulating the values that guide each party's interest in the process; (9) establishing and enlarging a common sense of purpose; (10) establishing formal rules about how decisions are reached; (11) establishing ground rules for conduct, and formally assessing the fairness of these from time to time; (12) inventorying all technical, financial, and human resources accessible to the collaborators; (13) negotiating agreement

among collaborators; (14) deciding how to implement and monitor the agreement; and (15) creating a local constituency to support implementation.

While this approach may be especially useful in promoting social learning in collaborative watershed planning exercises, with or without government involvement, the broader process of social learning in the debating and building of public policy may be better illustrated in Reich's (1988) discussion of "civic discovery." Reich envisions government, or an objective third party, as a catalyst for policy building and problem-solving excercises. The catalyst could get all the options, their benefits, and drawbacks on the table and generate a process of civic discovery. It is through such public deliberation about what is good for society that opinions can be revised, premises altered, and common interests discovered. Reich identifies at least four kinds of discovery that may ensue from such public exchanges:

(i) The problem and its solution may be redefined. People may discover that their initial assumptions about the nature of the problem and alternative solutions are wrong or inappropriate. Conflict may be redefined as a project almost everyone can support, even if it was or is not their preferred outcome.

(ii) Voluntary action may be generated. Consideration of the plight of those affected may lead others to volunteer time and money toward helping them adjust to the solution. Their willingness to volunteer stems from the discovery that others are also willing to help. Without public deliberation, individuals or groups might not discover that they can voluntarily help remedy the situation. They might assume that their charitable impulses are not widely shared and that their individual acts would be futile. Discovery empowers people to take voluntary action together.

(iii) Preferences may be legitimized. People may discover there are many others who share their values about the public good, even though these values have not been adequately represented publicly by selected interest groups.

(iv) Individual preferences may be influenced by a consideration of what is good for society or the resource. If individuals and groups debate a policy outside the narrow confines of their own interests and lobbying efforts, and if they are required to make public their reasons for supporting a policy, they may be influenced by the process.

If these types of social learning or civic discovery were integrated with the findings of institutional economists and anthropologists (e.g., Ostrom 1990, Pinkerton 1991) and expressed as progressive steps, the following would be likely to apply.

(*i*) Through discussion of available information, participants would define the major problems that have to be tackled and agree to work on these problems together.

(*ii*) Participants would openly discuss and debate alternatives about how best to solve the problems, and the roles they each might play in the solutions.

(*iii*) Participants would make public commitments about what each party is willing to contribute to the solution, after a thorough airing of alternatives.

(*iv*) Participants would work with a co-ordinator who is highly experienced in group process and interest-based planning and could assist them in identifying their significant shared interests and their mutual stake in the health of local stocks.

(*v*) Ideally (especially at the regional level) the co-ordinator would help stakeholders begin to work together by getting them involved in a series of small co-operative projects. This experience of working together would both develop some trust and understanding among participants, and also provide an early focus on practical, achievable tasks that illustate the benefits of all groups "putting the resource first." The experience of smaller projects would allow the groups to begin to see their mutual dependence in concrete terms, and to move toward taking on larger and longer range tasks.

(*vi*) The local management board should ideally be willing to set up a system of low-cost mutual monitoring by the parties or monitoring by a mutually acceptable party accountable to all. Such a system would assist parties in developing trust in management information and a sense of mutual accountability. Government authorities would need initial involvement to be satisfied that the system was workable, and would need longer term involvement in troubled areas.

The Role of the Department of Fisheries and Oceans

Obviously government would need to be involved in the type of changes discussed above, and could even be the sponsor and catalyst for the beginning of the process. However, government cannot be both the sponsor and the convenor, or the process will simply follow government's agenda, and fail to invoke trust and commitment from all parties. If government is involved, what is needed is a process and a structure managed from outside government, within which parties can work with each other and with government.

Fortunately, there are models both in Canada and the United States for how such a process can be successfully executed. In 1987 Labour Canada funded two of its former employees to set up an independent Public Policy Forum in Ottawa to bring government and the private sector together to identify and deal with contentious issues. Since then the Forum has assisted in setting up sectoral councils that take on issues in a more objective fashion than government or any sector can alone. Government usually contributes at least part of the funding to initiate the process, but the successful processes become mostly self-funding in a short time (Public Policy Forum 1993). A similarly independent policy forum to discuss fisheries management issues in British Columbia was initiated in 1993 by a former senior DFO employee and his associates. Housing itself at the Fisheries Centre at the University of British Columbia as the neutral forum, this Common Ground Project brings together perspectives from commercial fishers, sport fishers, First Nations, DFO, provincial fish management agencies, and the university. The group has formed an interim council and is planning its third meeting at this writing (Common Ground Project 1993).

In the United States, governments at all levels have experimented with various forms of contracting certain types of service delivery or policy-making roles to third parties: non-governmental organizations, branches of local government, and private contractors. Often government sets general guidelines within which these organizations work, but lets them do the job as best they see fit. A team including a 20-yr veteran of such experiments (Osborne and Gaebler 1993) claims that such partnerships deliver consistently better results at lower costs.

The example from their book most quoted by U.S. Vice President Gore in launching a 1993 U.S. federal program

by the same title, "Reinventing Government", concerns how garbage collection was made more efficient in Phoenix, Arizona. In response to a tax revolt, the city government decided to open one district of the city at a time to bids on a 5-yr garbage collection contract for that district, allowing the city's Public Works Department to submit bids alongside outside contractors. To avoid layoffs of public employees, the city required private contractors to hire displaced Public Works employees, and transferred those who wished to remain in Public Works to other jobs. After losing the first four contracts, Public Works was forced to reformulate more efficient and effective strategies. Over a 10-yr period it won back all the contracts and a higher morale among employees (Osborne and Gaebler 1993, p. 76–79). The example illustrates one of many mechanisms discussed by the authors for increasing accountability of government through creating incentives for delivery of appropriate service.

If salmon management did become more accountable, based on the development of policy and support by the fishing community, DFO would presumably continue to play a key role in the management system, in at least the following ways:

(*i*) DFO could be the party with ultimate responsibility for resource conservation and protection. It could reserve the right to intervene if it believed conservation was being compromised. Conservation standards and management objectives, goals, and procedures for specific areas could be spelled out in agreements between DFO and local bodies. All parties would be accountable to the agreement. As a practical interim arrangement, DFO could work closely with local boards until it was confident that procedures were running smoothly. Changes to annual management plans for each area could be negotiated annually.

(*ii*) DFO could be the provider of technical scientific expertise and advice, at least in the beginning. This means DFO would identify problems it sees and make data publicly available so others could verify that the problems were real. As technical expert, it would be important that DFO openly acknowledge the unknowns, uncertainties, and risks involved in any current or past policy or policy initiative. The fishing sectors and public should be asked to take responsibility for some of the risks in supporting a particular path of action.

(*iii*) DFO could be the catalyst (but not the co-ordinator or manager) of public and cross-sectoral discussion of the basic policy direction of management. This discussion is not about technical issues, but concerns what basic values and goals should drive the management system. In assisting in the development of a fundamental policy direction, the convenors should pay particular attention to fishing-dependent communities, the majority of which are not represented by current fishing organizations.

Conclusion

This discussion has examined the theory and the practice of community-based self-management and co-management in the light of its capacity to address the problems that plague the managers of common pool resources, such as dealing with the conditions of nonexcludability and subtractibility. Three types of elements key to the success of locally based regimes were identified: logistical, cost sharing, and power sharing. The social learning processes by which government and local bodies could move toward such regimes were also discussed.

Current managers, aboriginal peoples, and fishing sectors will eventually have to deal with these basic conditions, as well as the biological, economic, and political problems particular to the B.C. salmon fishery, if there is to be a resource in the future. This paper has explored various models and examples of multiparty co-management of fisheries, and suggested how elements of them could be applicable to the west coast salmon situation, helping to address the longstanding problems of this fishery. It was argued that co-management arrangements could contribute to improved data collection, analysis, and monitoring; they could lower the cost of fishing through more localized fisheries and lower the cost of management through cost recovery supported local management activities and organized volunteer efforts. Co-management arrangements could also contribute to the resolution of conflict among parties, and to the development of greater credibility of, participation in, and support for the management system. The analysis of existing cases suggests that such applications are possible, desirable, and could be part of a logical progression toward greater power sharing between DFO and fishing groups, as well as among different groups and sectors.

This progression would best begin with the public discussion and development of basic policy objectives for salmon management. The setting of goals and methods for achieving these objectives would then inform the process of deciding how the problems of the salmon fishery were approached. With a sense of greater security and predictability in clear policy objectives, fishing-dependent communities, as well as fishing organizations, could more openly consider the type of institutional possibilities discussed in this paper. The best supported and most effective policies and policy instruments are likely to be those that are thoroughly discussed and developed at the grass roots level. The institutions and processes for power sharing discussed in this paper are intended to suggest options that could be helpful in these discussions. One or several of these options, or combinations thereof, could be tried on an experimental basis by communities or regions interested in pursuing management improvements of the type described here. If some strategy for improvement is not developed, the fishery will be eroded by the combined biological, economic, and political problems previously identified.

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References

ACHESON, J. 1975. The lobster fiefs: economic and ecological effects of territoriality in the Maine lobster industry. Hum. Ecol. 3: 183–207.

ACHESON, J. 1988. The lobster gangs of Maine. University of New England Press, Hanover, N.H. 181 p.

AKAHA, T. 1993. Japanese–Russian fishery joint ventures and operations: opportunities and problems. Mar. Poli. 1993: 199–212.

ALBRECHT, D.E. 1990. Co-management as transaction: the Kuskokwim River salmon management working group. M.A. thesis, McGill University, Montreal, Que. 139 p.

ALVERSON, D.L. AND P.A. LARKIN. 1993. Fisheries: fisheries science and management—century 21, p. 150–167. *In* C.W. VOIGTLANDER [ed.] The state of the world's fisheries resource. Proceedings of the World's Fisheries Congress, Plenary Sessions. Oxford & IBH Publishing Co., PVT Ltd., Oxford & New Delhi.

ARGYRIS, C., AND D. SCHON. 1978. Organizational learning: a theory of action perspective. Addison-Wesley, Reading, Mass. 344 p.

AMEND, D.A. 1989. Alaska's regional aquaculture associations: co-management of salmon in southern southeast Alaska, p. 125–134. *In* E. PINKERTON [ed.] Co-operative management of local fisheries. New directions for improved management and community development. University of British Columbia Press, Vancouver, B.C.

BEFU, H. 1980. Political ecology of fishing in Japan: techno-environmental impact of industrialization in the Inland Sea. Res. Econ. Anthropol. 3: 323–347.

BERKES, F. 1981. The role of self-regulation in living resource management in the north, p.166–177. In M.M.R. FREEMAN [ed.] Renewable resources and the economy of the north. ACUNS/MAB, Ottawa, Ont.

BERKES, F., R. PRESTON, AND P. GEORGE. 1991. The evolution in theory and practice of the joint administration of living resources. Alternatives 18: 12–18.

BISSON, P.A., T.P. QUINN, G.H REEVES, AND S.V. GREGORY. 1992. Best management practices, cumulative effects, and long-term trends in fish abundance in Pacific Northwest river systems, p. 189–232. *In* R.J. NAIMAN [ed.] Watershed management. Balancing sustainability and environmental change. Springer-Verlag, New York, N.Y.

BLOMQUIST, W. 1992. Dividing the waters: governing groundwater in southern California. ICS Press, San Francisco, Calif. 409 p.

BOWLES, F., AND M. BOWLES. 1989. Holding the line: property rights in the lobster and herring fisheries of Matinicus Island, Maine, p. 228–257. *In* J. CORDELL [ed.] A sea of small boats. Cultural Survival Inc., Cambridge, Mass.

BROMLEY, D. 1989. Economic interests and institutions: the conceptual foundations of public policy. Basil Blackwell, New York, N.Y. 274 p.

BROWN, L.D. 1991. Bridging organizations and sustainable development. Hum. Rel. 44: 807–831.

BROWN, D. 1993. A critical look at DFO's reforms. Fisherman 58: 14

BRUUN, R. 1982. The Boldt decision: legal victory, political defeat. Law Pol. Q. 4: 271–298.

CARRIER, J., AND A. CARRIER. 1989. Marine tenure and economic reward on Ponam Island, Manus Province, p. 94–120. *In* John CORDELL [ed.] A sea of small boats. Cultural Survival, Inc, Cambridge, Mass.

CIRIACY-WANTHRUP, S.V., AND R.C. BISHOP. 1975. "Common property" as a concept in natural resource policy. Nat. Resour. J. 15: 713–727.

COHEN, F. 1986. Treaties on trial. The continuing controversy over northwest indian fishing rights. With contributions by Joan La France and Vivian Bowden. University of Washington Press, Seattle, Wash. 229 p.

COMMITINI, S. 1966. Marine resource exploitation and management in the economic development of Japan. Econ. Dev. Cult. Change. 14: 414–427.

COMMON GROUND PROJECT. 1993. The Common Ground Project prospectus and annexes. Fisheries Centre, University of British Columbia, Vancouver, B.C. 9 p.

CRUICKSHANK, D. 1991. A commission of inquiry into licencing and related policies of the Department of Fisheries and Oceans. T.D. Mock, Victoria, B.C. 113 p.

DALE, N. 1989. Getting to co-management: social learning in the redesign of fisheries management, p. 49–72. *In* E. PINKERTON [ed.] Co-operative management of local fisheries: new directions for improved management and community development. University of British Columbia Press, Vancouver, B.C.

DEPARTMENT OF FISHERIES AND OCEANS (DFO). 1988. Pacific region salmon stock management plan. I Inner south coast and Fraser River. Discussion Document 1986. Department of Fisheries and Oceans, 555 West Hastings, Vancouver, B.C. V6B 5G2. 204 p.

DEPARTMENT OF FISHERIES AND OCEANS (DFO). 1991. Report on the Central Coast salmon stock management plan (SSMP) process. Program Planning and Economics Branch, Pacific Region, Department of Fisheries and Oceans, 555 West Hastings, Vancouver, B.C. V6B 5G2. 36 p.

DEPARTMENT OF FISHERIES AND OCEANS (DFO). 1993a. Salmon expectations. Central coast. Pacific Region, Department of Fisheries and Oceans, 555 West Hastings, Vancouver, B.C. V6B 5G2. 191 p.

DEPARTMENT OF FISHERIES AND OCEANS (DFO). 1993b. West Coast Fisheries, changing times. Aboriginal Fisheries. Department of Fisheries and Oceans, Stations 314, 555 West Hastings St., Vancouver, BC V6B 5G3

DOBYNS, D. 1992. Watershed negotiations in the Nooksack River basin: a co-operative evaluation with a tribal perspective. M.S. thesis, Western Washington University, Bellingham, Wash. 60 p.

DYER, C.L., AND J.R. MCGOODWIN [ed.]. 1994. Folk management in the world's fisheries: lessons for modern fisheries management. University Press of Colorado, Niwot, Colo. 347 p.

FEENY, D., F. BERKES, B. MCCAY, AND J. ACHESON. 1990. The tragedy of the commons: twenty-two years later. Hum. Ecol. 18: 1–19.

FRASER, G.A. 1979. Limited entry: experience of the British Columbia salmon fishery. J. Fish. Res. Board Can. 36: 754–763.

GILHOUSEN, P. 1990. Prespawning mortalities of sockeye salmon in the Fraser River system and possible causal factors. Bull. 26: International Pacific Salmon Fisheries Commission. Vancouver, B.C. 58 p.

GRAY, B. 1985. Conditions facilitating interorganizational collaboration. Hum. Rel. 38: 911–936.

GRAY, B. 1991. Collaborating: finding common ground for multi-party problems. Jossey-Bass, San Francisco, Calif. 329 p.

GREER, A. 1993. Local salmon management. A proposal for co-operative, community-based management of Canada's Pacific salmon resource. Department of Fisheries and Oceans, 555 West Hastings, Vancouver, B.C. V6B 5G3. 25 p.

GROOT, C., K. DENMAN, K. HYATT, G. JAMIESON, AND D. WARE. 1992. Global climate change: British Columbia fisheries in a changing environment. Pacific Biological Station, Nanaimo, B.C. 12 p.

HARDIN, G. 1968. The tragedy of the commons. Science (Wash., D.C.) 162: 1243–1248.

HAYWARD, B. 1981. The B.C. salmon fishery: a consideration of the effects of licensing. B.C. Stud. 50: 39–51.

HENDERSON, M.A., D.A. LEVY, AND J.S. STOCKNER. 1992. Probable consequences of climate change on freshwater production of Adams River sockeye salmon. GeoJournal. 28: 51–59.

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE (IDRC). 1991. Tomorrow's catch. IDRC Reports. 19: 27 p.

IWAKIRI, S. 1983. Mataquali of the sea—a study of the customary right on reef and lagoon in Fiji, the South Pacific. Mem. Kagoshima Univ. Res. Cent. South Pac. 4: 133–143.

JENTOFT, S., AND T. KRISTOFFERSEN. 1989. Fishermen's co-management: the case of the Lofoten fishery. Hum. Organ. 48: 355–365.

JOHANNES, R.E. 1978. Traditional marine conservation methods in Oceania and their demise. Annu. Rev. Ecol. Syst. 9: 349–364.

JOHANNES, R.E. 1981. Words of the lagoon. University of California Press, Berkeley, Calif. 245 p.

JOHANNES, R.E. 1988. Research on traditional tropical fisheries: some implications for Torres Strait and Australian aboriginal fisheries, p. 30–41. *In* F. GRAY AND L. ZANN [eds.] Traditional knowledge of the marine environment in northern Australia. Great Barrier Reef Marine Park Authority, Townsville, Australia.

KALLAND, A. 1981. Shingu. A study of a Japanese fishing community. Scand. Inst. Asian Stud. Monogr. Ser. 44: 198 p.

KEARNEY, J.F. 1983. Common tragedies: a study of resource access in the Bay of Fundy herring fisheries. M.A. thesis, Dalhousie University, Halifax, N.S. 441 p.

KLEE, G.A. [ed.]. 1980. World systems of traditional resource management. John Wiley & Sons, New York, N.Y. 290 p.

KNAPP, G. 1992. Alaska salmon markets and prices. Institute of Social and Economic Research, University of Alaska, Anchorage, Alaska. 50 p.

KOFINAS, G., AND J. GRIGGS. 1993. Collaboration and the B.C. Round Table: an early-stage analysis of a "better way" of deciding. *In* T. GUNTON AND M. ROSELAND, [eds.] Alternative dispute resolution and public land planning: the Canadian experience. (Unpublished manuscript, available from 2208 Cyress St., Vancouver, V6J 3M5.) 53 p.

KRAVANJA, M. 1992. Russia–Japan hatchery projects. National Marine Fisheries Service, Silver Springs, Md. 12 p.

LARKIN, P. 1991. The wet revolution. Fisheries production to the year 2025. Address to the American Fisheries Society Workshop, May 1991, 21–23. Snowbird, Utah. University of British Columbia, 2204 Main Mall, Vancouver, B.C. 35 p.

LAWSON, P. 1993. Cycles in ocean productivity, trends in habitat quality and restoration of salmon runs in Oregon. Fisheries. (Bethesda) 188: 6–10.

LEE, R., AND I. DEVORE [ed.]. 1968. Man the hunter. Aldine, Chicago, Ill. 415 p.

LEVIEL, D.P. AND B. ORLOVE. 1990. Local control of aquatic resources: community and ecology in Lake Titicaca, Peru. Am. Anthropol. 92: 362–382.

LEVY, D.A. 1992. Potential impacts of global warming on salmon production in the Fraser River watershed. Fraser River Environmentally Sustainable Task Force. Can. Tech. Rep. Fish. Aquat. Sci. 1889. 96 p.

LICHATOWICH, J. 1994. Components of management baselines. Paper presented at "Pacific Salmon and their Ecosystems", Seattle, WA. 117-C Don Schmith, Sequim, WA 98382. 32 p.

MARCHAK, P.M., N. GUPPY AND J. MCMULLIN [ed.]. 1987. Uncommon property. The fishing and fish processing industries in British Columbia. University of British Columbia Press, Vancouver, B.C. 402 p.

MARTIN, F. 1989. Common pool resources and collective action: a bibliography. Vol. 1. Workshop in Political Theory and Policy Analysis, Indiana University, Bloomington, Ind. 310 p.

MARTIN, F. 1992. Common pool resources and collective action: a bibliography. Vol. 2. Workshop in Political Theory and Policy Analysis, Indiana University. Bloomington, Ind. 140 p.

MCCAY, B.J. 1989. Sea tenure and the culture of the commoners, p. 203–227. *In* J. CORDELL [ed.] A sea of small boats. Cultural Survival, Inc., Cambridge, Mass.

MCCAY, B.J. AND J. ACHESON [ed.]. 1987. The question of the commons: the culture and ecology of communal resources. University of Arizona Press, Tucson, Ariz. 439 p.

MCEVOY, A.F. 1986. The fisherman's problem: ecology and law in the California fisheries, 1850–1980. Cambridge University Press, New York, N.Y. 368 p.

MCGOODWIN, J.R. 1990. Crisis in the World's Fisheries: People, Problems, and Policies. Stanford University Press, Stanford, Calif. 235 p.

NEHLSEN, W., J.E. WILLIAMS, AND J.A. LICHATOWICH. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries (Bethesda) 16: 4–21.

NETBOY, A. 1968. The atlantic salmon. A vanishing species? Houghton Mifflin, Boston, Mass. 457 p.

NETBOY, A. 1973. The salmon. Their fight for survival. Houghton Mifflin, Boston, Mass. 613 p.

NETTING, R. 1976. What alpine peasants have in common: observations on communal tenure in a Swiss village. Hum. Ecol. 4: 135–146.

NORTHCOTE, T.G., AND D.Y. ATAGI. 1994. Pacific salmon abundance trends in the Fraser River watershed compared with other British Columbia systems. Paper read at the conference Pacific Salmon and Their Ecosystems. Jan. 10–12, 1994, Seattle, Wash. Department of Fisheries and Oceans, 610 Derwent Way, Annacis Island, New Westminster, B.C. V3M 5P8. 26 p.

OSBORNE, D., AND T. GAEBLER. 1992. Reinventing government: how the entrepreneurial spirit is transforming the public sector. Addison-Wesley, New York, N.Y. 405 p.

OSTROM, E. 1987. Institutional arrangements for resolving the commons dilemma: some contending approaches, p. 250–265. *In* B. MCCAY AND J. ACHESON [ed.] The question of the commons: the culture and ecology of communal resources. University of Arizona Press, Tucson, Ariz.

OSTROM, E. 1990. Governing the commons. The evolution of institutions for collective action. Cambridge University Press, Cambridge, UK. 280 p.

PAVEL, J. 1989. The Puget Sound salmon management plan: a co-operative management tool, p. 22–29. *In* B.L. SMITH AND P. HURT [ed.] Fisheries co-management: a response to legal, social and fiscal imperatives. Great Lakes Indian Fish and Wildlife Commission, Odanah, Wis.

PEARSE, P. 1982. Turning the tide. A new policy for Canada's Pacific fisheries. Final Report. The Commission on Pacific Fisheries Policy, Department of Fisheries and Oceans, 555 West Hastings, Vancouver, B.C. V6B 5G3. 292 p.

PINKERTON, E.W. 1988. Co-operative management of local fisheries: a route to development, p. 257–273. *In* J.W. BENNETT AND J.R. BOWEN [ed.] Production and autonomy. Anthropological studies and critiques of development. Monogr. Econ. Anthropol. 5.

PINKERTON, E.W. 1989. Introduction. Attaining better fisheries management through co-management—prospects, problems, and propositions. *In* E.W. PINKERTON [ed.] Cooperative management of local fisheries: new directions for improved management and community development. University of British Columbia Press, Vancouver, B.C.

PINKERTON, E.W. 1991. Locally based water quality planning: contributions to fish habitat protection. Can. J. Fish. Aquat. Sci. 48: 1326–1333.

PINKERTON, E.W. 1992. Translating legal rights into management practice: overcoming barriers to the exercise of co-management. Hum. Organ. 51: 330–341.

PINKERTON, E.W. 1994a. Summary and conclusions, p. 317–337. *In* C.L. DYER AND J.R. MCGOODWIN [ed.] Folk management in the world's fisheries. University Press of Colorado, Niwot, Colo.

PINKERTON, E.W. 1994b. Economic and management benefits from the co-ordination of capture and culture fisheries: the case of Prince William Sound pink salmon. N. Am. J. Fish. Manage. 14: 262–277.

PINKERTON, E.W., AND N. KEITLAH. 1990. The Point No Point Treaty Council: innovations by an inter-tribal fisheries management cooperative. School of Community and Regional Planning and Nuu-chah-nulth Tribal Council, UBC Centre for Human Settlements, 2206 East Mall, Vancouver, B.C. V6T 1W5. 54 p.

PINKERTON, E.W. AND S. LANGDON. 1987. Regional aquaculture associations in Alaska: an overview, p. 1–23. *In* E. PINKERTON, F. COHEN, S. LANGDON, N. KEITLAH, W. REES, P. BOOTHROYD, D. LIGHTLY, AND B. GREEN [ed.] Launching a fisheries co-management pilot project: learning from the experience of Alaska and Washington State. Phase I. Final Report. Department of Fisheries and Oceans, Indian and Northern Affairs, B.C. Native Affairs Secretariat. 555 West Hastings, Vancouver, B.C. V6B 5G3. 23 p.

PINKERTON, E.W., D. MOORE, AND F. FORTIER. 1993. A model for First Nation leadership in multi-party stewardship of watersheds and their fisheries. Report to the Royal Commission on Aboriginal Peoples. Ottawa, Ont. 26 p.

PITT, D. 1993. The overfishing crisis: there's ample cause for global alarm. Vancouver Sun, Vancouver, B.C. August 7: B4

PUBLIC POLICY FORUM. 1993. Making government work. 130 Albert St. Ottawa, ON K1P 5G4. 23 p.

REICH, R. 1988. Policy-making in a democracy, p. 123–156. *In* R. Reich, [ed.] The power of public ideas. Ballinger, Cambridge, Mass.

RIDDELL, B. 1993. Spatial organization of Pacific salmon: what to conserve? p. 23–41. *In* J. G. CLOUD AND G. H. THORGAARD [ed.] Genetic conservation of salmonid fishes. NATO ASI Ser. A.

RUDDLE, K. 1989. Solving the common property dilemma: village fisheries rights in Japanese coastal waters, p. 168–184. *In* F. BERKES, [ed.] Common property resources. Ecology and community-based sustainable development. Belhaven Press, London.

RUDDLE, K., AND T. AKIMICHI [ed.]. 1984. Maritime institutions in the western pacific. National Museum of Ethnology, Osaka, Japan. 329 p.

RUDDLE, K., AND R.E. JOHANNES [ed.] 1985. The traditional knowledge and management of coastal systems in Asia and the Pacific. UNESCO, Jakarta Pusat, Indonesia. 313 p.

SEILER, D., P. HANRATTY, S. NEUHAUSER, P. TOPPING, M. ACKLEY, AND L. KISHIMOTO. 1992. Wild salmonid production and survival evaluation. Washington Department of Fisheries, P.O. Box 43138, Olympia, WA 98504-2700. 40 p.

SEITER, A.E. 1993. Putting process into practice: tribal leadership in regional water planning. Paper for the American Water Resource Association Symposium. 27–30 June 1993. Seattle, Wash. Jamestown S'Klallam Tribe, 1103 Old Blyn Highway, Sequim, WA 98382. 12 p.

SHORT, K. 1989. Self-management of fishing rights by Japanese co-operative associations: a case study from Hokkaido, p. 371–387. *In* J. CORDELL [ed.] A sea of small boats. Cultural Survival, Inc., Cambridge, Mass.

SMITH, C. 1994. Connecting cultural and biological diversity in restoring northwest salmon. Fisheries (Bethesda) 19: 20–26

STOFFLE, B.W., D.B. HALMO, R.W. STOFFLE, AND G.G. BURPEE. 1994. Folk management and conservation ethics among small-scale fishermen of Buen Hombre, Dominican Republic, p. 115–138. *In* C.L. DYER AND J.R. MCGOODWIN [ed.] Folk management in the world's fisheries. University Press of Colorado, Niwot, Colo.

TAYLOR, G. 1993. Proposal for a new salmon management regime on the Skeena River. M.A. thesis. Simon Fraser University. Burnaby, B.C. 75 p.

WALTERS, C.J. 1988. Mixed-stock fisheries and the sustainability of enhancement production for chinook and coho salmon, p. 109–115. *In* W.J. MCNEIL [ed.] Salmon production, management, and allocation: biological, economic, and policy issues. Oregon State University Press. Corvallis, Oreg.

WALTERS, C.J., R.D. GORUK AND D. RADFORD. 1993. Rivers Inlet sockeye salmon: an experiment in adaptive management. N. Am. J. Fish. Manage. 13: 253–262

WIGEN, K. 1989. Shifting control in Japan's coastal waters, p. 388–410. *In* J. CORDELL [ed.] A sea of small boats. Cultural Survival, Inc., Cambridge, Mass.

WITHLER, F.C. 1982. Transplanting Pacific salmon. Can. Tech. Rep. Fish. Aquat. Sci. 1079: 27 p.

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